




EX LIBRIS
UNIVERSITATIS
ALBERTENSIS

The Bruce Peel
Special Collections
Library



Digitized by the Internet Archive
in 2025 with funding from
University of Alberta Library

<https://archive.org/details/0162014920456>

University of Alberta

Library Release Form

Name of Author: **Alison Margaret Derry**

Title of Thesis: **Zooplankton Communities and Genetic Divergence of Rotifers in Saline and Subsaline Lakes**

Degree: **Master of Science**

Year Degree Granted: **2001**

Permission is hereby granted to the University of Alberta Library to reproduce single copies of this thesis and to lend or sell such copies for private, scholarly, or scientific research purposes only.

The author reserves all other publication and other rights in association with the copyright in the thesis, and except as herein before provided, neither the thesis nor any substantial portion thereof may be printed or otherwise reproduced in any material form whatever without the author's prior written permission.

**The phenomenal populations of rotifers are similar to
the number of stars in our galaxy.**

(T. Cavalier-Smith, 1982.)

University of Alberta

**ZOOPLANKTON COMMUNITIES AND GENETIC DIVERGENCE OF
ROTIFERS IN SALINE AND SUBSALINE LAKES**

by

ALISON MARGARET DERRY



A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment
of the requirements for the degree of MASTER OF SCIENCE

in

ENVIRONMENTAL BIOLOGY AND ECOLOGY

DEPARTMENT OF BIOLOGICAL SCIENCES

Edmonton, Alberta
Fall, 2001

University of Alberta

Faculty of Graduate Studies and Research

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled, **ZOOPLANKTON COMMUNITIES AND GENETIC DIVERGENCE OF ROTIFERS IN SALINE AND SUBSALINE LAKES** submitted by **ALISON MARGARET DERRY** in partial fulfillment of the requirements for the degree **MASTER OF SCIENCE** in **ENVIRONMENTAL BIOLOGY AND ECOLOGY.**

To the Water Ghosts Whose Memories Will Always Bring Me Back

ABSTRACT

Although salinity and aquatic biodiversity are inversely related in lake water, the relationship between types of salts and zooplankton communities is poorly understood. Further, there is no information on whether variation in salt concentration contributes to the genetic divergence of zooplankton populations. In my study, lake water dominated by chloride anions had distinct zooplankton communities from those dominated by sulphate/carbonate anions. This distinction likely resulted from the combined effects of contrasting water chemistries and predation regimes. Greater haplotype diversity and genetic divergence was observed among populations of halophilic *Brachionus plicatilis* than among populations of predominantly freshwater *Keratella quadrata* rotifers. The most divergent *B. plicatilis* population was a strain that was most abundant at lower salinities. I provide preliminary evidence for an additional sibling species in the *B. plicatilis* species complex. This study documents some of the first molecular phylogenetic work conducted on rotifers.

ACKNOWLEDGEMENTS

I would like to thank my supervisors, Dr. Paul Hebert and Dr. Ellie Prepas, for their interest, support, and encouragement in this project. My supervisory committee, Dr. Allen Good, Dr. Vince St. Louis, and Dr. Janusz Zwiazek also provided helpful guidance.

I am thankful to Shannon Corrigan and Lucille Kowalchuk for their patience and dedicated help in the field during the quest for saline lakes and rotifers, and for their tolerance of our vessel, the *Rotifer Hunter*. Maria Sotiropoulos and Clayton Saffron also helped us on various field expeditions. The wardens, in particular Glynnis Hood and Libby Gunn, at the Fort Smith office in Wood Buffalo National Park provided invaluable assistance. I am also grateful to Aurora College, Fort Smith, N.W.T. for providing lab space and the use of microscopes, as well as living quarters. The staff at the Environmental Unit at Sycrude Canada Ltd. also allowed our use of their equipment, office space, resources, and facilitated our access to Saline Lake, north of Fort McMurray.

I greatly appreciate the friendship, support, and technical guidance that I received from members of the Hebert lab at the University of Guelph: Sarah Adamowitz, Andrea Cox, Melania Cristiceau, Jeremy deWaard, Rob Dooh, Ryan Gregory, Dave Hardie, Dr. Paul Hebert, Dr. Ed Remigio, James Rydderich, Dr. Veronika Sacherova, and Jonathan Witt. Members of the Prepas lab at the University of Alberta also provided academic feedback and moral support, in particular Erik Allen, Nicole Armstrong, Theo Charette, Devin Millions, Paula Siwik, Kim Westcott, and Carol Nahorniak. Kara Vlasman and Cherie Westbrook also gave invaluable moral support to me.

Patricia Burgess, Brian Rolseth, and Roselyn Rudy from the limnology laboratory at the University of Alberta analyzed many of the water chemistry samples. Emma Mangas from the laboratory of Dr. Pinel Alloul at the Université de Montréal did the final analysis of zooplankton samples. Dr. Richard Stemberger at Dartmouth College, New Hampshire provided verification of certain rotifer identifications. DNA was submitted to Angela Holliss at the Guelph Molecular Supercenter for sequencing. I am also grateful to Dr. Felix Sperling for allowing me the use of his MacIntosh computers at the University of Alberta to run phylogenetic analyses. Dr. Chantal Ouimet provided advice on the use of multivariate statistics for zooplankton community analysis.

Financial support was provided by a number of different agencies that included the Biodiversity Challenge Grants program through the Alberta Conservation Association, Syncrude Canada Ltd., the Alberta Sport, Recreation, Parks and Wildlife Foundation, Northern Scientific Training Grants, and NSERC operating grants to E.E. Prepas and P.D.N. Hebert. Wood Buffalo National Park and Aurora College, Fort Smith, N.W.T. provided in-kind support.

I am especially grateful for the love and support of my family and Mike.

TABLE OF CONTENTS

Chapter 1: Introduction	
1.0 Project Rationale.....	1
1.1 Objectives.....	2
1.2 References.....	4
Chapter 2: Environmental Determinants of Zooplankton Communities in Saline and Subsaline Lakes.	
2.0 Introduction.....	6
2.1 Methods and Materials.....	8
2.1.1 Description of Study Lakes.....	8
2.1.2 Field Collection and Laboratory Analysis.....	10
2.1.3 Statistical Analysis.....	13
2.3 Results.....	16
2.3.1 Lake Morphometry and Water Chemistry.....	16
2.3.2 Biota.....	18
2.3.3 Species-Environment Relations.....	20
2.3.3.1 Subsaline Lakes.....	20
2.3.3.2 Salt Lakes with Chloride-dominated Water.....	21
2.3.3.3 Salt Lakes with Chloride- versus Sulphate/Carbonate-dominated Water.....	22
2.4 Discussion.....	25
2.5 References.....	46
Chapter 3: Evolution of Monogonont Rotifers: a Molecular Phylogenetic Approach	
3.0 Introduction.....	54
3.0.1 Phenotypic Variation Among Species.....	54
3.0.2 Genetic Divergence Within Species.....	55
3.0.3 Application of Molecular Systematics.....	56
3.1 Materials and Methods.....	58
3.1.1 Field Collection and Identifications.....	58
3.1.2 Molecular Protocols.....	59
3.1.3 Sequence and Phylogenetic Analysis.....	59
3.2 Results.....	64
3.2.1 16S: Inter-familial and Inter-specific Comparisons of Selected Taxa.....	64
3.2.1.1 Sequence Variability.....	64
3.2.1.2 Phylogenetic Analyses.....	64
3.2.2 COI: Inter and Intra-specific Comparisons of Selected Taxa.....	65
3.2.2.1 Sequence Variability.....	65
3.2.2.2 Phylogenetic Analyses.....	66
3.2.3 COI: Intraspecific Comparisons within <i>Brachionus plicatilis</i>	70
3.2.3.1 Sequence Variability.....	70
3.2.3.2 Phylogenetic Analyses.....	70

3.3 Discussion.....	72
3.3.1 Between-gene Comparisons.....	72
3.3.2 Phenotypic Plasticity Among Species.....	73
3.3.3 Genetic Divergence Within Species.....	75
3.4 References.....	91
 Chapter 4: General Conclusions	
4.0 General Conclusions.....	98
4.1 References.....	101
 Appendix A.....	103
 Appendix B.....	106
 Appendix C.....	107
 Appendix D.....	108
 Appendix E.....	109
 Appendix F.....	110
 Appendix G.....	111
 Appendix H.....	112
 Appendix I.....	113
 Appendix J.....	114
 Appendix K.....	121
 Appendix L.....	130

LIST OF TABLES

Table 2-1. The location and morphometry of saline and subsaline study lakes. At the end of the lake designations, -SO ₄ ²⁻ refers to the SO ₄ ²⁻ /CO ₃ ²⁻ -dominated saline waters in central Alberta, and -Cl and -D respectively refer to Cl ⁻ -dominated saline waters and subsaline waters found in the northern part of the province.....	35
Table 2-2. Measurements of TDS (mg/l), conductivity (cond., µS/cm), major nutrient (µg/l) and ion (mg/l) concentrations for the study lakes over summer of 1999. pH, DOC (mg/l), turbidity (NTU), colour (mg/l Pt), and chl <i>a</i> (µg/l) are also presented. SO ₄ ²⁻ -dominated saline waters in central Alberta (-SO ₄) were measured in only June. Averages for June, July, August and September were calculated for Cl ⁻ -dominated saline waters (-Cl) and subsaline waters (-D), with the exception of SL-Cl that was sampled only in June and August. NO ₂ and NO ₃ (µg/l) were measured in July for most of the lakes, only once in August for SL-Cl, and n.d. indicates where no data was available. Salinity categories are given according to Hammer (1986a): hypersaline is >50 000 mg/l TDS, mesosaline is 20 to 50 000 mg/l TDS, and hyposaline is 3 to 20 000 mg/l TDS.....	36
Table 2-3. Salinity categories (total dissolved solids (TDS), Hammer 1986a), and summer measurements of conductivity (cond., µS/cm), total phosphorus (TP, µg/l), ions (mg/l), pH, and chl <i>a</i> (µg/l) for the saline study lakes located in central Alberta. Values are averaged for June, July and August from 1983 to 1992 for PN-SO ₄ and FL-SO ₄ (Evans 1995) and from 1983 to 1984 for OL-SO ₄ (Bierhuizen and Prepas 1985).....	37
Table 2-4. Peak density observed for rotifer species (# individuals/l lake water) in each category of lake-water salinity (Hammer 1986a) over the summer of 1999. Dominant anions for each lake category are indicated in brackets.....	38
Table 2-5. Peak density observed for crustacean species (# individuals/l lake water) in each category of lake-water salinity (Hammer 1986a) over the summer of 1999. Dominant anions for each lake category are indicated in brackets	39
Table 2-6. List of zooplankton species present in saline lakes containing Cl ⁻ -dominated waters, but that were not included in the CCA of all saline study lakes because of the restricted number of species that could be included in the analysis. <i>Brachionus quadridentatus</i> , <i>Keratella quadrata</i> , and <i>Daphnia parvula</i> were included in the CCA, and were positively correlated with the following list of taxa that were not included in the CCA.....	40

Table 3-1. Number of haplotypes per rotifer species (haplotype names correspond with notation for COI sequences), number of replicate sequences per haplotype and locations of sample collection for 16S sequences.....	82
Table 3-2. Mean decimal percent 16S divergence (\pm SE) between phylogenetic clusters of rotifers identified by NJ (Appendix E).....	83
Table 3-3. Number of haplotypes per rotifer species, number of individuals sequenced per haplotype, and locations of sample collection for COI sequences. <i>Brachionus plicatilis</i> was collected from saline waters > 3000 mg/l total dissolved solids (HC, GB, SP, and FP lakes). All other lakes were subsaline (500 to 3000 mg/l total dissolved solids).....	84
Table 3-4. Mean decimal percent COI divergence (\pm SE) among rotifer species identified by NJ (Appendix F and G).....	85

LIST OF FIGURES

Figure 2-1. The location of study lakes in Wood Buffalo National Park and Alberta, Canada in relation to the distribution of the difference between precipitation and open water evaporation in North America..... 41

Figure 2-2. Depth profiles of conductivity indicating inverse stratification of water in (A) subsaline FP-D and (B) mesosaline HC-Cl. This phenomenon was displayed in September in FP-D and throughout the summer in HC-Cl. Surface areas of FP-D and HC-Cl were 3.3 and 4.4 ha, respectively..... 42

Figure 2-3. Ordination biplots of (A) species and environmental variables, and (B) sites and environmental variables from partial RDA of rotifer species in the subsaline study lakes. In (A), species ordination positions are represented by numbers:
1. *Testudinella patina*, 2. *Cohurella obtusa*, 3. *Lecane luna*,
4. *Monostyla quadridentus*, 5. *Notommata* sp., 6. *Notholca acuminata*, 7. *Keratella quadrata* and *Trichotria tetractis*.
8. *Lepadella patella*, 9. *Lophocharis salpina*, 10. *Anuraeopsis fissa*, 11. *Asplanchna brightwelli* and *Ascomorpha ecaudis*,
12. *Monostyla closterocerca* and *Monostyla lunaris*,
13. *Asplanchna priodonta*, *Collotheca pelagica*, *Encentrum* sp., *Keratella valga*, *K. hiemalis*, and *Trichocerca multicrinis*,
14. *Keratella ticinensis* and *Trichotria pocillum*,
15. *Brachionus plicatilis*, *Filinia longiseta*, *Hexarthra* sp.,
16. *Keratella testudo* and *Pompholyx* sp., 16. *Lepadella acuminata*,
17. *Keratella cochlearis*, 18. *Gastropus stylifer*, *Trichocerca lophoessa*, 19. *Vanoyella globosa*, 20. *Trichocerca rattus*,
21. *Ascomorpha ovalis*, 22. *Mytilina ventralis* var. *brevispina*,
22. *Platytias platytias*, 24. *Polyarthra vulgaris*, 25. *Lecane ohioensis*, 26. *Brachionus quadridentatus*, 27. *Synchaeta* sp.,
28. *Polyarthra dolichoptera*, 29. *Keratella serrulata*,
29. *Cohurella uncinata*, *Trichocerca longiseta*, 31. *Monostyla bulla*..... 43

Figure 2-4. Ordination biplots of (A) species and environmental variables, and (B) sites and environmental variables from partial RDA of rotifer and crustacean zooplankton species within the saline lakes with Cl⁻-dominated water. In (B), the filled symbols represent samples from the mesosaline lakes and empty symbols represent the hyposaline lakes. In (A), filled symbols indicate species found only in the mesosaline lakes, empty symbols are species found only in the hyposaline lakes, and black-and-white symbols are species that appeared in both mesosaline and hyposaline study lakes..... 44

Figure 2-5. Ordination biplots of (A) species and environmental variables, and (B) sites and environmental variables from partial CCA of rotifer and crustacean zooplankton found in Cl⁻-dominated (circles) and SO₄²⁻/CO₃²⁻-dominated (squares) saline lake water. Of the Cl⁻-dominated waters in (A), species found at hyposaline concentrations are represented by empty symbols, species found at mesosaline concentrations are solid circles, and species found at both meso- and hyposaline concentrations are black-and-white circles. Species found only in the SO₄²⁻/CO₃²⁻-dominated lake water are indicated by solid squares in (A). Zooplankton species that appeared in both lakewater ion types are indicated by solid diamonds. Abbreviations: A_o= surface area and Alk = total alkalinity (HCO₃⁻ + CO₃²⁻)..... 45

Figure 3-1. The location of saline (GB, SP, FP and HC) and subsaline (GL, BP, WR, GW and near Fort McMurray) lakes in Wood Buffalo National Park and Alberta, Canada from which rotifer DNA was sequenced..... 85

Figure 3-2. Seventy-percent majority rule concensus of 2 equally parsimonious trees for the 15 16S rotifer sequences rooted with *Asplanchna* sp. Bootstrap support (1000 pseudoreplicates) for major clades is indicated above the nodes. Numbers at the terminal branches give the haplotype and correspond with the numbering assigned to equivalent COI taxa. Capitalized letter clades indicate clusters representing different genera: A = *Brachionus* sp., B = *Keratella* sp., and C = *Synchaeta* sp..... 86

Figure 3-3. Seventy-percent majority rule concensus of 50 equally parsimonious trees of the 42 COI rotifer nucleotide sequences rooted with *Asplanchna* sp. Bootstrap support (1000 pseudoreplicates) for major clades is indicated above the nodes. Numbers at the terminal branches give the nucleotide haplotype and correspond with the numbering assigned to equivalent 16S taxa. Capitalized letter clades indicate clusters representing different genera: A = *Brachionus* sp., B = *Keratella* sp., C = *Synchaeta* sp. Lowercase letter clades indicate clusters of *Brachionus plicatilis* haplotypes, which correspond to the lakes from which the animals were collected: clades a and b = HC Lake, clade c = FP Lake, and clade d = GL/SPL lakes. These clades also corresponded with amino acid haplotypes in Appendix G. Spined (S) and unspined (NS) subspecies of *Keratella cochlearis* as well as two-spined (2S) and one-spined (1S) morphs of *K. hiemalis* are indicated on the figure..... 87

Figure 3-4. Seventy-percent majority rule consensus of 856 equally parsimonious trees of the 37 COI *Brachionus plicatilis* nucleotide sequences rooted with *B. calyciflorus*. Bootstrap support (1000 pseudoreplicates) for major clades is indicated above the nodes. Accession numbers AF266855 to AF266950 belong to the 21 haplotypes identified by Gomez et al (2000b) in the Iberian Peninsula, Spain. *B. plicatilis* -1 to -16 are haplotypes found in Wood Buffalo National Park (WBNP), Canada. Numbers at the terminal branches give the nucleotide haplotype for WBNP populations and correspond with the numbering assigned on other 16S and COI trees..... 88

Figure 3-5. Neighbour-joining phenogram of 37 COI *Brachionus plicatilis* amino acid sequences based on uncorrected p-distances and rooting with *B. calyciflorus*. Accession numbers AF266855 to AF266950 belong to the 21 haplotypes identified by Gomez et al (2000b) on the Iberian Peninsula, Spain. *B. plicatilis* -1 to -16 are haplotypes found in Wood Buffalo National Park (WBNP), Canada. Numbers at the terminal branches give the nucleotide haplotype for WBNP populations and correspond with the numbering assigned on other 16S and COI trees. Lowercase letter clades indicate clusters of WBNP *B. plicatilis* haplotypes, which correspond to lakes from which the animals were collected: clades a and b = HC Lake, clade c = FP Lake, and clade d = GL/SPL lakes..... 89

CHAPTER 1: INTRODUCTION

1.0 Project Rationale

Lakes with elevated salinity may become increasingly abundant as a result of climate change (e.g., Hammer 1990, Covich et al. 1997) and anthropogenic activities, such as irrigation in arid regions (e.g., deVillers 1999) and oil sand mining (Environment Canada 1998). Increased lakewater salinity is a concern because it is associated with decreased diversity of zooplankton, an important component of aquatic food webs (Williams et al. 1990, Hammer 1990, Frey 1993, Hammer 1993, Williams 1993). In addition to salt concentration, the ionic composition of salts in lake water can alter zooplankton communities (e.g., Frey 1993, Bos et al. 1996, Williams 1998, Bos et al. 1999). Sulphate/carbonate-dominated salts are the most common type of lakewater salinity in North America, and most salt lake research on this continent has focused on these systems. In contrast, chloride-dominated saline lake water is comparatively rare in North America and has received less attention. Identifying relationships between environmental variables associated with salinity and zooplankton species assemblages will clarify how planktonic animals are distributed among saline aquatic environments with contrasting water chemistry.

Lakewater salinity may also influence zooplankton at the population level by altering evolutionary rates of DNA sequence divergence. Accelerated rates of molecular evolution have been reported for saline lake fauna, such as brine shrimp (*Artemia franciscana*) (Iwabe et al. 1996, Maley and Marshall 1998) and halophilic cladocerans (Hebert 1998, Hebert et al. 2001). In addition, salinity is thought to increase intraspecific diversity of *Daphnia pulex* in coastal ponds that receive evaporites from seaspray

(Weider and Hebert 1987, Wilson and Hebert 1992). Rotifers of the class Monogononta (Appendix A) are often abundant in saline lakes (Hammer 1993). This rotifer class is thought to have high rates of speciation because of their short generation times and their cyclic parthenogenetic mode of reproduction, with alternating cycles of asexual and bisexual reproduction (Serra et al. 1997). The water in salt lakes is frequently subject to extreme fluctuations in salinity (Hammer 1986). This temporal variation may influence the timing of sexual reproduction and enhance intraspecific genetic differentiation of rotifers across geographic areas (DeMeester 1996, Gomez et al. 2000, Serra et al. 1997). In addition to understanding how changes in lakewater salinity may affect the community composition of zooplankton, exploring the genetic divergence of rotifer populations in a range of saline waters will contribute to our understanding of the relationship between habitat gradients and species diversification.

1.1 Objectives

The overall objective of this thesis was to describe how zooplankton species and populations are distributed among lake water with different salinities. Chapter 2 focuses on communities of crustaceans and rotifers found in lakes spanning a salinity gradient, while Chapter 3 tackles the population divergence of rotifer species that were abundant in these lakes. In Chapter 2, the primary objective was to evaluate differences in zooplankton communities between chloride- and sulphate-dominated waters. A secondary objective was to evaluate the relationship between zooplankton community composition and ion chemistry in subsaline lakes. Chapter 3 addresses genetic divergence among rotifer populations from subsaline and chloride-dominated saline

lakes. While predominantly freshwater *Keratella quadrata* was most abundant in the subsaline lakes, halophilic *Brachionus plicatilis* was confined to saline waters. My hypothesis was that there would be greater genetic diversity among populations of the halophilic species compared to predominantly freshwater *K. quadrata*. This prediction was based on the greater potential for cryptic speciation of halophiles in the absence of morphological change at different salinities in salt lakes. A secondary objective of Chapter 3 was to resolve species boundaries between several *Keratella* spp. that have variable posterior spine length, a characteristic that can confuse taxonomic designations. Chapters 2 and 3 provide insight into the unique communities and populations of zooplankton that inhabit extreme environments such as saline lakes. Learning about saline lakes that span a gradient of salt concentration and a range of brine type provides an opportunity to understand how salinity contributes to the spatial structuring of aquatic communities and populations.

1.2 References

- Bos, D.G., B.F. Cumming and J.P. Smol. 1999. Cladocera and Anostraca from the Interior Plateau of British Columbia, Canada, as paleolimnological indicators of salinity and lake level. *Hydrobiologia* 392: 129-141.
- Bos, D.G., B.F. Cumming, C.E. Watters and J.P. Smol. 1996. The relationship between zooplankton, conductivity and lake-water ionic composition in 111 lakes from the Interior Plateau of British Columbia, Canada. *International Journal of Salt Lake Research* 5: 1-15.
- Covich, A.P., C. Fritz, P.J. Lamb, R.D. Marzolf, W.J. Matthews, K.A. Poiani, E.E. Prepas, M.B. Richman and T.C. Winter. 1997. Potential effects of climate change on aquatic ecosystems of the Great Plains of North America. *Hydrological Processes* 11: 993-1021.
- DeMeester, L. 1996. Local genetic differentiation and adaptation in freshwater zooplankton populations: patterns and processes. *Ecoscience* 3: 385-399.
- deVillers, M. 1999. *Water*. Stoddart Publishing Company Ltd., Toronto. 413 pp.
- Environment Canada. 1998. Research Priorities for Aquatic Resources in the Oilsands. Presented by Kevin Cash (regulatory agency) at Conrad Environmental Aquatics Technical Advisory Group (CEATAG) Workshop. Nov. 9-10, 1998, Calgary, AB. (Available from Syncrude Canada Ltd.).
- Frey, D.G. 1993. The penetration of cladocerans into saline waters. *Hydrobiologia* 267: 233-248.
- Gomez, A., G.R. Carvalho and D.H. Lunt. 2000. Phylogeography and regional endemism of a passively dispersing zooplankter: mitochondrial DNA variation in rotifer resting eggs. *Proceedings of the Royal Society of London* 267: 2189-2197.
- Hammer, U.T. 1986. *Saline Ecosystems of the World*. Dr. W. Junk Publishers, Dordrecht. 616 pp.
- Hammer, U.T. 1990. The effects of climate change on the salinity, water levels and biota of Canadian prairie saline lakes. *Verhandlungen Internationale Vereinigung für Limnologie* 24: 321-326.
- Hammer, U.T. 1993. Zooplankton distribution and abundance in saline lakes of Alberta and Saskatchewan, Canada. *International Journal of Salt Lake Research* 2: 111-132.

- Hebert, P.D.N. 1998. Variable environments and evolutionary diversification in inland waters. In: G.R. Carvalho (ed.). *Advances in Molecular Ecology*. IOS Press, Amsterdam. Pp. 267-290.
- Hebert, P.D.N., E.A. Remigio, J.K. Colbourne, D.J. Taylor and C.C. Wilson. 2001. Accelerated molecular evolution in halophilic crustaceans. Submitted.
- Iwabe, N., K. Kuma and T. Takashi. 1996. Evolution of gene families and relationship with organismal evolution: rapid divergence of tissue-specific genes in the early evolution of chordates. *Molecular Biology and Evolution* 13: 483-493.
- Maley, L.E. and C.R. Marshall 1998. The coming of age of molecular systematics. *Science* 279: 505-506.
- Serra, M., A. Galiana and A. Gomez. 1997. Speciation in monogonont rotifers. *Hydrobiologia* 358: 63-70.
- Weider, L.J. and P.D.N. Hebert. 1987. Ecological and physiological differentiation among low-arctic clones of *Daphnia pulex*. *Ecology* 68: 188-198.
- Williams, W.D., A.J. Boulton and R.G. Taaffe. 1990. Salinity as a determinant of salt lake fauna: a question of scale. *Hydrobiologia* 197: 257-266.
- Williams, W.D. 1993. The worldwide occurrence and limnological significance of falling water-levels in large, permanent saline lakes. *Verhandlungen Internationale Vereinigung für Limnologie* 25: 980-983.
- Williams, W.D. 1998. Salinity as a determinant of the structure of biological communities in salt lakes. *Hydrobiologia* 381: 191-201.
- Wilson, C.C. and P.D.N. Hebert. 1992. The maintenance of taxon diversity in an asexual assemblage: an experimental analysis. *Ecology* 73: 1462-1472.

CHAPTER 2: ENVIRONMENTAL DETERMINANTS OF ZOOPLANKTON COMMUNITIES IN SALINE AND SUBSALINE LAKES

2.0 Introduction

Saline lakes comprise almost half of the global volume of inland surface water (Vallentyne 1972) and are systems with naturally low biodiversity (Hammer 1986a). The distinction between saline and subsaline waters varies with the composition of brine and occurs at 1000 mg/l total dissolved solids (TDS) for sulphate-dominated waters (Bierhuizen and Prepas 1985) and at 3000 mg/l TDS for chloride-dominated waters (Hammer 1986a). Inland saline lakes can be found on every continent where climatic and geologic conditions allow salts to concentrate. Common examples include greater evaporation than precipitation and basin water retention that allows for the accumulation of salts derived from soils in the drainage basin, sea spray, or wind-blown dust. Less commonly, saline lakes are derived from the solution of ancient marine deposits (Hammer 1986a).

Brine composition can be variable among saline lake water and is related to geologic setting and lake position in the drainage basin relative to local and regional groundwater flow (Last 1992). Most Canadian lakes that contain water with elevated salinities are located in the prairies and aspen parklands of Saskatchewan and Alberta, and in the dry interior of British Columbia. Lake water in these regions are usually dominated by sulphate or bicarbonate/carbonate anions, and vary with respect to cation dominance (Rawson and Moore 1944, Topping and Scudder 1977, Last and Schweyen 1983, Hammer 1986b, Bierhuizen and Prepas 1985, Renaut 1990, Last 1992, Blinn 1993, Wilson et al. 1994, Bos et al. 1996, Evans and Prepas 1996, Covich et al. 1997). With

the exception of Siberian saline systems (Zotina et al. 1999), sulphate-dominated saline waters in North America have unique water chemistry (Hammer 1986a). In contrast, sodium chloride dominance is the most common form of lakewater salinity globally, and is most frequently encountered in Australia and South Africa (Hammer 1986a). In North America, lake water dominated by sodium chloride salts tend to be found in arid regions of the western United States (Blinn 1993). There are a few isolated examples of sodium chloride-dominated lake water in Canada (Hammer 1986a), and several of these are located on the predominantly freshwater Boreal Plain (Camsell 1917, Tsui 1982).

Zooplankton species found in saline lakes are believed to be of freshwater origin (Beadle 1969). The diversity and species richness of salt lake zooplankton rapidly decline as osmotic tolerances are exceeded with increasing salinity (Williams et al. 1990, Hammer 1990, Frey 1993, Hammer 1993, Williams 1993). However, this trend is less obvious between waters of different salinity than the transition from freshwater to saline, and depends on the scale of salinity being compared (Williams et al. 1990, Williams 1998). In addition to salinity, ionic composition is an important determinant of the zooplankton species present (Hammer 1986a, Frey 1993, Bos et al. 1996, Williams 1998, Bos et al. 1999). Hammer (1993) reported that depth, pH, transparency, temperature and time of sampling also determine zooplankton communities in Canadian saline lakes. Although rotifers are often a common component of saline waters (Wurtsbaugh and Berry 1990, Hammer 1993, Leland and Berkas 1998, Zotina et al. 1999), most studies have focused on crustacean zooplankton (e.g. Reynolds 1979, Galat and Robinson 1983, Williams et al. 1990, Ivanova 1990, Hammer and Forró 1992, Frey 1993, Evans et al. 1995, Bos et al. 1996, Bos et al. 1999).

The primary objective of this study was to identify differences in zooplankton communities between chloride- and sulphate-dominated lake water, and determine whether variation in zooplankton distribution is related to water chemistry associated with salinity. We tested the hypothesis that differences in communities were largely a result of different anion dominance rather than overall salinity. Much of the emphasis was placed on unstudied saline lakes containing water dominated by sodium chloride because these systems have received little scientific attention in Canada. Additionally, we investigated which environmental variables explained variation in zooplankton distribution among northern subsaline lakes. Most studies of salt lakes have focused on crustacean zooplankton, and we attempt to relate differences in ion composition to the relative abundance of both crustaceans and rotifers. Our study takes place in a context where anthropogenic salinization of surface water is increasing and is recognized as an international threat to water quality (de Villiers 1999).

2.1 Methods and Materials

2.1.1 Description of Study Lakes

The study was conducted primarily on nine lakes in northern Alberta (Figure 2-1) during the summer of 1999. Three saline lakes in central Alberta (Figure 2-1) were also sampled in the spring and combined with data from Bierhuizen and Prepas (1985), Campbell and Prepas (1986), and Evans and Prepas (1996) for comparison with the northern lakes. At the end of abbreviations for the study lakes, -SO₄ identifies the sulphate-dominated saline lakes, -Cl refers to the chloride-dominated saline lakes, and -D indicates the more dilute subsaline lakes.

The study lakes are found in a 750 km band stretching from the northern tip of the Province of Alberta to southeast of the City of Edmonton. Eight of the study lakes (GB-Cl, HC-Cl, SP-Cl, GL-D, GW-D, BP-D, FP-D, and WR-D) are found on the Boreal Plain in Wood Buffalo National Park (WBNP), and are part of a World Heritage Site (Canadian Parks Service 1992). These study sites are located 0.5 to 45 km west of the Slave River in the north-eastern corner of the park (Figure 2-1). Boreal mixed-wood forest is interdispersed with wetlands, prairies, and salt flats (Larsen 1997, Moser et al. 1998). Also on the Boreal Plain is study lake SL-Cl, which is located 330 km south of the WBNP sites at La Saline Natural Area (Alberta Environmental Protection 1998), near Fort McMurray (Figure 2-1). In contrast, the three saline study lakes in central Alberta (OL-SO₄, PN-SO₄, and FL-SO₄) are located 150 km southeast of Edmonton (Figure 2-1) in aspen prairie-parkland, and are surrounded by farmland (Campbell and Prepas 1986).

The bedrock geology of the WBNP lakes is characterized by Middle Devonian limestone (CaCO₃), gypsum (CaSO₄•2H₂O), and dolostone (CaMg(CO₃)₂) shale, covered with a thin layer of glacial, glacial-lacustrine, lacustrine and aeolian deposits (Lemmen et al. 1994). Surface waters are influenced to varying degrees by deep, groundwater springs that discharge sodium chloride salt (Camsell 1917, Tsui 1982) from along the dissolution edge of the Cold Lake Formation of marine evaporitic halite (NaCl) (Mejer Drees 1986). SL-Cl is located near the boundary where limestone, gypsum and dolomite deposits meet Lower Cretaceous sandstone and minor shale (Government and the University of Alberta 1969). The discharge of sodium chloride springs into SL-Cl is periodically diluted when the nearby (0.2 km) Athabasca River floods (M. MacKinnon, Syncrude Canada Ltd., pers. comm.). In contrast, study lakes in central Alberta have a bedrock geology

comprised of Upper Cretaceous deposits of sandstone, shale, coal and bentonite (Government and the University of Alberta 1969). Dominant SO_4^{2-} -based minerals in the prairies and aspen parklands include gypsum, mirabilite ($\text{Na}_2\text{SO}_4 \cdot 10\text{H}_2\text{O}$) and thenardite (Na_2SO_4), and common carbonate-based minerals are aragonite (CaCO_3), calcite (CaCO_3), and dolomite ($\text{CaMg}(\text{CO}_3)_2$) (Last and Schweyen 1983). Feldspars containing K^+ are also abundant (Last and Schweyen 1983).

All of the study lakes are found in regions in which lake evaporation exceeds precipitation (Figure 2-1). The difference between precipitation and open water evaporation is approximately negative 15 cm for the nine lakes in northern Alberta on the Boreal Plain and approximately negative 35 cm for the three lakes located in central Alberta on the aspen-prairie parklands (Winter 1989).

2.1.2 Field Collection and Laboratory Analysis

During the summer of 1999, the three saline (GB-CI, HC-CI, SP-CI) and five subsaline (GL-D, GW-D, BP-D, FP-D, WR-D) lakes in WBNP were sampled monthly from June to September. During the same summer, SL-CI near Fort McMurray, AB was sampled once in June and August. The three study sites in central Alberta (OL- SO_4 , PN- SO_4 , FL- SO_4) were sampled once in June, 1999. With the exception of SL-CI, all sampling was conducted at the point of maximum depth, which was determined by hand-sounding transects across the lakes in an inflatable boat. The detail to which depth transects could be conducted at SL-CI was restricted by the method of access and values for maximum and mean depths are estimates for this lake. Lake surface area was determined with a Bioquant HPI digitizer with Bioquant System IV image analysis software and 1:50 000 topographic maps. BP-D was too small to be found on a map, and

since this pond was approximately circular, surface area was estimated based on measurements of length and width. Water transparency was estimated with a Secchi disk. Temperature and conductivity of surface lake water were measured in the field with a YSI 30 Conductivity Meter (Yellow Springs Instruments, Yellow Springs, OH), and pH was measured with a hand-held Fisher pH/temperature meter 119 Model 3D (Fisher Scientific, Pittsburgh, PA). Depth profiles at maximum depth were measured for temperature and conductivity in the WBNP saline and subsaline lakes in September.

Water samples were taken at approximately 0.5 m below the lake surface with acid-washed and pre-rinsed polyethylene bottles, and were refrigerated from 1 to 10 d before being shipped on ice for filtration and analysis by the Limnology Laboratory at the University of Alberta, Edmonton. Nitrite and nitrate samples were collected in July from saline and subsaline lakes in WBNP, and in August from SL-C1. These samples were then frozen within several hours of collection and analyzed in Edmonton within 5 d of sampling. Water was filtered for chlorophyll *a* (chl *a*) analysis with Gelman GF/C (0.7 μ m pore) filters, and the filters were wrapped in foil and frozen on desiccant beads in light resistant containers until analysis.

Water samples were analyzed for total dissolved solids (TDS), conductivity, total phosphorus (TP), total nitrogen (TN), nitrite (NO_2^-) and nitrate (NO_3^-), sodium (Na^{2+}), calcium (Ca^{2+}), potassium (K^+), magnesium (Mg^{2+}), iron (Fe^{2+}), manganese (Mn^{3+}), chloride (Cl^-), sulphate (SO_4^{2-}), alkalinity (CaCO_3 , HCO_3^- and CO_3^{2-}), dissolved organic carbon (DOC), turbidity and colour. The amount of TDS present in the water was determined according to Stainton et al. (1977), and conductivity was measured again in the laboratory with a Radiometer/Copenhagen Model CDM 83 conductivity meter (Bach-

Simpson Ltd., London, ON). TP was determined following the potassium persulfate digestion procedures of Menzel and Corwin (1965), as modified by Prepas and Rigler (1982). TN, and NO_2^- and NO_3^- were analyzed with a Technicon II autoanalyzer by automated Cu/Cd reduction (Armstrong et al. 1967), with modifications based on U.S. EPA Method 353.2. Cations were measured with a Perkin Elmer 3300 Atomic Absorption Spectrometer according to Stainton et al. (1977) and anion concentrations were determined with a Dionex 2000i/SP Ion Chromatograph according to Pfaff (1993). Alkalinity (CaCO_3 , HCO_3^- , and CO_3^{2-}) was analyzed with a Mettler DL21 Titrator with Mettler ST20 Sample Changer according to Greenberg et al. (1992). Turbidity was measured with a Hach Turbidimeter Model 2100A and colour was determined according to Cuthbert and del Giorgio (1992). Chl *a* was measured following the chlorophyll extraction method described by Winternmans and DeMots (1965) as modified by Bergmann and Peters (1980).

Plankton hauls were conducted each time water was sampled from the lakes. Zooplankton were sampled with a conical 64 μm tow net with a 20 cm diameter and a 50 cm length from near the bottom to the surface of each lake during daylight. Three vertical tows per sample were taken in lakes with maximum depths greater than or equal to 1 m. Horizontal drift tows were taken in lakes with maximum depths less than 1 m, and distances were estimated based on measurements taken from shore. Zooplankton were preserved in a chilled 4% buffered formalin-sucrose solution described by Prepas (1978). Taxonomic identifications and counting were conducted on 5 to 10 ml subsamples with a counting wheel on a dissecting microscope at 50X and 100X magnifications. Rotifers were identified according to Edmondson (1959), Chengalath et

al. (1971), and Stemberger (1979). Smith and Fernando (1978) was used to identify adult copepods, and the keys used for cladoceran identification included Edmondson (1959), Brooks (1957), Brandlova et al. (1972), and Devey and Devey (1971). Despite recent advances in systematics (Colbourne and Hebert 1996), *Daphnia pulex* and *D. pulicaria* were counted as a single taxonomic unit because of difficulty in morphologically distinguishing the two species (Dodson 1981). Estimated densities of zooplankton were expressed in number of individuals per litre, with the assumption that the filtration efficiency of the net was 100%. Indices of Shannon diversity and species richness were calculated for samples from the study lakes. Nauplii and other unidentifiable juveniles (mostly copepods) were excluded from zooplankton community analyses. Submerged aquatic vegetation gathered from the lakes was identified according to (Burland, G.R., undated) and Nelson and Paetz (1992) was employed to identify fish that were caught with minnow traps and then released.

2.1.3 Statistical Analysis

Sigmastat 2.0 for Windows (SPSS Inc. 1997) was employed to conduct ANOVAs and Spearman Rank correlations. Two-way repeated measures ANOVAs were conducted to detect differences ($P < 0.05$) in comparisons of diversity and richness indices. Spearman Rank correlations were performed to detect significant relationships ($P < 0.05$) between diversity indices and environmental variables among the 1999 samples ($n=35$). Spearman Rank correlations were also employed to determine significant relationships among species ($P < 0.05$) to determine how the dataset could be collapsed to meet sample size restrictions imposed by multivariate analyses.

Multivariate analyses were performed with CANOCO 4.0 (ter Braak and Šmilauer 1998) to investigate species-environment relations among the study lakes. Comparisons were drawn among the subsaline study lakes ($n=18$ samples collected in 1999), among the Cl^- -dominated saline lakes ($n=13$ samples collected in 1999), and among all of the saline study lakes dominated by Cl^- or SO_4^{2-} ($n=25$ samples collected in 1984 and 1999). Detrended correspondence analysis (DCA) was first employed to determine whether unimodal (canonical correspondence analysis) or linear (redundancy analysis) models of species response to environmental variables best fit the data by determining the lengths of gradients. The length of the gradient is a measure of how unimodal the species responses are along an ordination axis (range of sample scores \div average within species standard deviation along the axis) (ter Braak and Šmilauer 1998). Canonical correspondence analysis (CCA) and redundancy analysis (RDA) are ordination techniques that are employed to infer how environmental variables collectively influence community composition, in which the ordination axes are constrained to be a linear combination of environmental variables (Jongman et al. 1987, ter Braak and Šmilauer 1998, Legendre and Legendre 1998). Whereas CCA is a unimodal model in which species scores are weighted averages with respect to the sample scores, RDA is a linear model in which species scores are derived from weighted linear regression of the species data on the sample scores. CCA with downweighting of rare species was applied to data with long gradients determined by DCA (>4.0 standard deviations), and RDA was applied to data with short gradients (<4.0 standard deviations).

The minimum number of measured environmental variables that could account for the major directions of variance in the species data was determined by forward-

selected CCA or RDA with 999 iterations in Monte Carlo permutation tests at $P < 0.05$, unless noted otherwise. For example, if a variable was close to the limit of significance (i.e., $P \leq 0.06$), it was included in the minimum set of environmental variables.

Environmental variables that were identified as significant by forward selection but that were strongly correlated with other variables in the minimum set ($P < 0.001$), such that variance inflation factors > 20 (ter Braak and Šmilauer 1998), were excluded to avoid collinearity. Time of sampling in Julian days was a covariate for time-series restricted permutations in all multivariate analyses. Monte Carlo permutation tests (999 iterations) were employed to determine whether ordination axes significantly explained variance in the species data ($P < 0.05$). The degree to which an environmental variable contributes to an ordination axis increases with the length of the vector and with the proximity of its alignment to the ordination axis (ter Braak and Šmilauer 1998).

Subsaline lakes were analyzed separately from saline lakes in the ordination analyses. Subtle differences in species distribution among the 13 to 170 times more dilute subsaline lakes could not be resolved by the ordination axes when subsaline lakes were analyzed with saline lakes. Additionally, the number of species that could be included in CCA was restricted by the number of sampling events (n) and number of environmental variables (q) to $< n - q$ (ter Braak and Šmilauer 1998). Rare species that were positively correlated with other more abundant species were therefore excluded in cases where CCA was employed. Crustaceans and rotifers were analyzed together in ordinations of saline lakes, but were analyzed separately in ordinations for subsaline lakes because the number of correlated species $> n - q$ for the CCA of all species combined.

2.3 Results

2.3.1 Lake Morphometry and Water Chemistry

All of the lakes had small surface areas (≤ 150 ha) and were shallow (< 3.4 m mean depth) (Table 2-1). With the exception of the deepest lake (GL-D), the subsaline lakes (-D) were up to 4 times deeper and the saline lakes with water dominated by SO_4^{2-} anions were up to 7 times deeper than the saline lakes with Cl^- -dominated water. Mean summer Secchi disk readings for the subsaline lakes were 3.7 m in GL-D, 1.7 m in GW-D, 1.4 m in BP-D, 0.71 m in FP-D, and 1.2 m in WR-D. The Secchi disk was visible on the bottom of all of the lakes with Cl^- -dominated water but not on the bottom of the lakes with $\text{SO}_4^{2-}/\text{CO}_3^{2-}$ -dominated water. June of 1999 Secchi disk readings were 0.65 m in OL- SO_4 , 0.55 m in PN- SO_4 , and 0.30 m in FL- SO_4 . My Secchi disk readings were 2.5 times, 2.3 times, and 1.6 times lower, respectively, than measurements taken for the same three - SO_4 lakes in June, 1983 and 1984. With the exception of GB- Cl and SP- Cl that have surface outflows but not inflows, all of the saline and subsaline study lakes in WBNP have inflowing and outflowing streams. In contrast, the lakes with SO_4^{2-} -dominated water in central Alberta do not have any permanent inflowing or outflowing streams.

With the exception of hypersaline OL- SO_4 ($> 50\,000$ mg/l TDS), the study lakes ranged from subsaline (500 to 3000 mg/l TDS) to hyposaline (3000 to 20 000 mg/l TDS) to mesosaline (20 000 to 50 000 mg/l TDS) (Hammer et al. 1986a) (Table 2-2). Brine composition was also variable among lakes (Table 2-2). Fluctuation in lakewater salinity occurred over the summer at all of the sites but, with the exception of FP-D, all of the

study lakes remained in the salinity categories given in Table 2-2. FP-D was hyposaline in mid-June but became subsaline by the end of the month after a major storm event (86 mm precipitation over 2 d, Wood Buffalo National Park 1999). However, although this lake was subsaline for most of the summer, the bottom stratum of water overlying the sediments became hyposaline by September (Figure 2-2). Further, mesosaline HC-Cl demonstrated inverse stratification with warmer, denser water on the bottom (Figure 2-2) throughout the summer. All other saline and subsaline study lakes in northern Alberta remained isothermal and of the three study lakes containing $\text{SO}_4^{2-}/\text{CO}_3^{2-}$ -dominated water in central Alberta, only the water column of OL- SO_4 exhibited inverse chemical stratification during 1984 (Campbell and Prepas 1986).

Ion composition varied dramatically among the study lakes (Table 2-2). With the exception of one subsaline lake with water dominated by SO_4^{2-} (GL-D), the other four subsaline lakes had water dominated by Cl^- and were located within 0.5 to 45 km of the saline study lakes in WBNP (Figure 2-1). Similarly, the four saline study lakes in northern Alberta had water dominated by Cl^- (3832 to 16 765 mg/l) and also had high concentrations of SO_4^{2-} (340 to 2219 mg/l). In contrast, Cl^- concentrations were 2 to 21 times lower and 12 to 37 times lower, respectively, than the SO_4^{2-} and $\text{CO}_3^{2-}/\text{HCO}_3^-$ anions that dominated lake water in the aspen-prairie parklands of central Alberta. Total alkalinity was comprised of CaCO_3 and HCO_3^- in Cl^- -dominated saline and subsaline lake water, and by CaCO_3 , HCO_3^- and CO_3^{2-} in the 28 to 68 times more alkaline $\text{SO}_4^{2-}/\text{CO}_3^{2-}$ -dominated lake water. With the exception of GL-D where Ca^{2+} prevailed, Na^+ was the most abundant cation in all of the subsaline and saline lake water, and was followed in

abundance by Ca^{2+} in Cl^- -dominated lake water and by K^+ in $\text{SO}_4^{2-}/\text{CO}_3^{2-}$ -dominated waters.

Concentrations of nutrients also varied between Cl^- - and $\text{SO}_4^{2-}/\text{CO}_3^{2-}$ -dominated lake water (Table 2-2). With the exception of GL-D that contained SO_4^{2-} -dominated oligotrophic water, the subsaline and saline lakes at the northern Alberta study sites had TP and TN concentrations that corresponded with mesotrophic levels of productivity for freshwater (Wetzel 1983). These lakes had concentrations of TN that exceeded TP, and TN:TP ranged from 12 to 57. In contrast, saline lakes in central Alberta had hyper-eutrophic levels of TP (Wetzel 1983) that were $>1322 \mu\text{g/l}$, and TN:TP ranged from 0.02 to 0.05. With the exception of the more productive FP-D and HC-Cl, chl α concentrations (Table 2-2) in the saline and subsaline study lakes in this study were indicative of mesotrophy (Wetzel 1983).

2.3.2 Biota

Dominant submerged aquatic vegetation in the subsaline lakes consisted of *Ceratophyllum demersum*, *Alisma gramineum*, *Utricularia vulgaris*, and filamentous green algae. Vast beds of *Chara* sp. were observed in GL-D. With the exception of SL-Cl that had similar plant life as the subsaline lakes, submerged aquatic vegetation found in the Cl^- -dominated saline lake water was different and usually less extensive than in subsaline waters. An unidentified benthic algae was observed in GB-Cl and SP-Cl. *Potamogetan pusillus* was identified in HC-Cl and emergent *Salicornia rubens* grew near the outlet of this lake. Plants were scarce in lake water dominated by $\text{SO}_4^{2-}/\text{CO}_3^{2-}$ and consisted of benthic *Rhizoclonium hieroglyphicum* in PN- SO_4 and FL- SO_4 and a filamentous green algae, possibly *Pithophora* sp., in OL- SO_4 (Campbell and Prepas

1986). Of the macro-invertebrates that were observed in the saline lakes, corixids were abundant in Cl^- -dominated waters and Campbell and Prepas (1986) reported that Ephydriidae larvae were prevalent in OL-SO_4 . With the exception of HC- Cl and FP-D, nine-spined stickleback (*Pungitius pungitius*) were detected in all of the WBNP saline study lakes. In contrast, fish were absent from the lakes with water dominated by $\text{SO}_4^{2-}/\text{CO}_3^{2-}$ (Campbell and Prepas 1986).

Zooplankton community composition contrasted between different concentrations and types of lakewater salinity (Tables 2-4 and 2-5). Peak densities for most crustacean and rotifer species were observed in the subsaline lakes. *Brachionus plicatilis** (Table 2-4) was absent from most subsaline lakes but occurred in extremely high numbers at the end of June in FP-D, which was shortly after water in this lake had changed from hyposaline to subsaline. The rotifers *Hexarthra* sp. and *B. plicatilis* thrived in the Cl^- -dominated hyposaline lake water. *B. plicatilis* and *Cletocamptus* sp. were found in high densities in mesosaline lake water dominated by Cl^- throughout the summer, and *Ceriodaphnia pulchella* occurred in lower numbers. In contrast, calanoid copepods, *Diaptomus nevadensis* and *Leptodiaptomus sicilis*, and rotifer *Brachionus urceolaris*, were most abundant in the mesosaline lake water dominated by $\text{SO}_4^{2-}/\text{CO}_3^{2-}$ in June. In hypersaline OL-SO_4 , rotifers were rare and *Artemia franciscana* was the predominant crustacean.

Zooplankton Shannon diversity and species richness indices were lower ($P=0.02$) in saline lakes (diversity = 0.39 ± 0.23 , species richness = 3.78 ± 2.53) than subsaline waters (diversity = 1.29 ± 0.20 , richness = 13.83 ± 2.19) in WBNP. Deepest GL-D was excluded from the analysis to reduce confounding effects of depth. Shannon diversity

and species richness were negatively correlated with TDS, conductivity, Cl^- , SO_4^{2-} , Na^+ , Ca^{2+} and K^+ , as well as pH and surface area, but were positively correlated with mean depth. The lowest zooplankton diversity was observed in mesosaline HC-Cl in September, which had a monoculture of *Brachionus plicatilis*.

2.3.3 Species-Environment Relations

2.3.3.1 Subsaline Lakes

Environmental gradients between subsaline lakes were less extreme than between saline lakes, and consequently, species-environment relationships were less clearly defined for rotifers and crustaceans than in the saline lakes. The minimum set of environmental variables that described the greatest variation in rotifer species distribution in the subsaline lakes was determined by forward-selected redundancy analysis (RDA), and included Cl^- and Mg^{2+} . Na^+ was also identified as a significant variable but was positively correlated with Cl^- ($r=0.99$), and therefore was not included in the analysis. Since forward selection identified pH as only marginally insignificant ($P=0.06$), this variable also included in the minimum set of environmental variables to be tested in the RDA. Axis one ($\lambda_1=0.25$) and axis two ($\lambda_2=0.11$) explained 26.3% and 11.7%, respectively, of the variation in species data. Axis one was most strongly influenced by Cl^- (inter-set correlation = 0.74) and Mg^{2+} (inter-set correlation= 0.51), and axis two was determined primarily by pH (inter-set correlation = 0.84) (Figure 2-3).

Rotifer species that ordinated to the right of the origin (0,0) along the Cl^- and Mg^{2+} vectors were strongly correlated with these elements (Figure 2-3A). While *Lecane luna* appeared beside the Mg^{2+} vector, *Colurella obtusa*, *Monostyla quadridentus*, *Notommata* sp., *Notholca acuminata*, *Keratella quadrata*, *Trichotria tetractis* and

Lepadella patella were strongly associated with Cl^- . Distribution patterns for species ordination 14 to 21 (Figure 2-3A) were not clear, but appeared to be correlated with less Mg^{2+} , which varied from 27 mg/l to 52 mg/l (Table 2-2). Species positions 22 to 21 (Figure 2-3A) were strongly associated with the pH axis and were more abundant at higher pH measurements, which ranged from 7.4 to 9.2. In contrast, species ordinations 9 to 13 appeared in the quadrant opposite to the pH vector and were most abundant at the lower end of this pH range.

For the crustacean zooplankton in the subsaline lakes, total alkalinity ($\text{CaCO}_3 + \text{HCO}_3 + \text{CO}_3$), SO_4^{2-} , Cl^- , pH and temperature comprised the minimum set of environmental variables, which was determined by forward-selected CCA. Na^+ ($r=0.98$), Mg^{2+} ($r=0.91$), and conductivity ($r=0.84$) were also identified as significant variables, but were correlated with Cl^- . K^+ was also an important parameter, but was correlated with total alkalinity ($r=0.78$). However, none of the axes explained variation in crustacean species distribution among the subsaline study lakes when the minimum set of significant variables was tested.

2.3.3.2 Salt Lakes with Chloride-dominated Water

Environmental variables that described variation in species data among lakes containing water dominated by Cl^- anions were determined by forward-selected RDA and included Cl^- , Na^+ , conductivity, and TDS. Since the latter three variables were strongly correlated with Cl^- ($r=0.94$, 0.92 , and 0.91 respectively), they were excluded from the analysis. Only axis 1 was significant for the RDA ($\lambda_1=0.28$, 28.6% of variance explained in species data). Cl^- was the sole environmental influence in the analysis and had an inter-set correlation of -0.70 to axis one (Figure 2-4).

Species found to the left of the origin were more strongly correlated with Cl^- than species located to the right of the origin (Figure 2-4A). *Brachionus plicatilis* occurred across the entire range of conductivity measured for saline lake water dominated by Cl^- (10 185 to 52 489 $\mu\text{S}/\text{cm}$), but was most abundant in mesosaline waters. *Cletocamptus* sp. occurred in some samples of only the mesosaline lakes (HC-Cl and GB-Cl) at amounts ranging from 5265 to 16 475 mg/l Cl^- . *Daphnia parvula*, *Keratella cochlearis* and *Bosmina* sp. were present in low densities (0.025 individuals/l) on one occasion in mesosaline HC-Cl. In contrast, species found to the right of the origin were most abundant in the hyposaline NaCl lakes (SP-Cl and SL-Cl). Many of these species were found in SL-Cl, the least saline of the salt lakes with water dominated by Cl^- (Table 2-4). The highest density of any one species was *Hexarthra* sp. (752 individuals/l), which occurred in the August sample of hyposaline SP-Cl (6116 mg/l Cl^-). Mesosaline (9716 to 20 827 mg/l Cl^-) and hyposaline (3190 to 6116 mg/l Cl^-) lake samples are shown in Figure 2-4B. Although differences in zooplankton communities did occur at different salt concentrations, the most abundant taxa were present across the salinity range studied.

2.3.3.3 Salt Lakes with Chloride- versus Sulphate/Carbonate-dominated Water

The set of environmental variables that described the greatest variation in the species data from all of the saline lakes was determined by forward-selected canonical correspondence analysis (CCA). This set was comprised of total alkalinity ($\text{HCO}_3 + \text{CO}_3$), SO_4^{2-} , Cl^- , Ca^{2+} , surface area, Na^+ , K^+ , conductivity, and TP. Within the set of variables identified by forward selection, strong correlations ($P > 0.001$) that raised variance inflation factors > 20 occurred between Cl^- and Na^+ ($r = 0.97$), Cl^- and conductivity ($r = 0.97$), total alkalinity and K^+ ($r = 0.91$), and between total alkalinity and

TP ($r=0.93$). Consequently, Na^+ , conductivity, K^+ and TP were removed from the analysis to minimize the number of environmental factors tested. CCA with the minimum set of five variables yielded two significant axes ($\lambda_1=0.97$ and $\lambda_2=0.62$) (Figure 2-5). Axis one was most strongly influenced by total alkalinity (inter-set correlation = 0.99), SO_4^{2-} (inter-set correlation = 0.79), and surface area (inter-set correlation = 0.66). This axis explained 35% of the species variance. Axis two was most strongly defined by Cl^- (inter-set correlation = 0.79) along with Ca^{2+} (inter-set correlation = -0.57), and explained 22.2% of species variance.

Contrasting trends were observed in the water chemistry of OL- SO_4 in 1983/84 and 1999. OL- SO_4 samples from 1983/84 ordinated beside the SO_4^{2-} vector when included in the CCA, and reflected the extremely high concentrations of SO_4^{2-} anions (29 422 mg/l) that year. In June 1999, however, SO_4^{2-} concentrations were closer to PN- SO_4 and FL- SO_4 , CO_3^{2-} was the dominant anion, and Cl^- concentrations were elevated (Table 2-2). OL- SO_4 was the only lake studied that contained water with hypersaline concentrations of salt, and differences among the other less saline lakes were difficult to discern when samples from OL- SO_4 were active in defining ordination axes. Consequently, OL- SO_4 was excluded from the CCA.

The CCA ordination diagram of taxa positions in Cl^- -dominated and $\text{SO}_4^{2-}/\text{CO}_3^{2-}$ -dominated saline lake water, excluding OL- SO_4 , indicates striking differences in zooplankton communities between these two lake types (Figure 2-5A). *Leptodiatomus sicilis* and *Diaptomus nevadensis* ordinated along the first axis and were strongly associated with alkaline conditions (2782 to 3020 mg/l $\text{HCO}_3^- + \text{CO}_3^{2-}$) and high concentrations of SO_4^{2-} (2525 to 7544 mg/l). *Daphnia similis* showed similar trends as *L.*

sicilis and *D. nevadensis* in 1984. *Brachionus urceolaris* was an outlier that was treated as a supplementary species and therefore, did not influence the definition of the ordination axes. *B. urceolaris* was found only in June, 1999 in PN-SO₄ and FL-SO₄, and the plotting of its position in the ordination diagram after the analysis indicated an affiliation with high concentrations of SO₄²⁻ (3387 to 7544 mg/l).

Cl⁻ and Ca²⁺ accounted for variation in species distribution in the second axis (Figure 2-5A). Harpacticoid copepod *Cletocamptus* sp. and rotifer *Brachionus plicatilis* ordinated beside the Cl⁻ vector and were strongly associated with sodium chloride (NaCl) salts. Also located along the second axis was *Hexarthra* sp., which was found in lakes with elevated concentrations of Cl⁻ (505 to 19 551 mg/l) and Ca²⁺ (13 to 986 mg/l) (Table 2-4). *Lophocharis salpina* was a rare species that appeared once in hyposaline SP-Cl, and *Daphnia parvula* and *Brachionus quadridentatus* were present in low numbers in some of the Cl⁻ and Ca²⁺-rich waters. Although *K. quadrata* did appear in both the SO₄²⁻/CO₃²⁻- and Cl⁻-dominated lakes, it was more abundant in lake water dominated by Cl⁻ (Table 2-4). The number of species that could be included in the CCA was restricted by the number of study lakes and the frequency of sampling (*n*=25 samples), as well as by the number of environmental variables tested (5) (ter Braak and Šmilauer 1998). Species that were positively correlated with *Daphnia parvula*, *Keratella quadrata*, and *Brachionus quadridentatus* that appeared in low densities in Cl⁻-dominated saline lake water were not included in the analysis (Table 2-6). These rarer species would have ordinated near their correlated counterparts in the CCA had they been included in the analysis and would not have changed the outcome of the ordination plots.

The site-environment biplot for saline lakes clearly resolved Cl^- -dominated lake water from $\text{SO}_4^{2-}/\text{CO}_3^{2-}$ -dominated waters (Table 2-2, Figure 2-5B). Mesosaline PN- SO_4 and FL- SO_4 clustered together along the first axis to the far right of the origin, and SL- Cl , HC- Cl and GB- Cl lakes ordinated above the origin to the left of the second axis. Although hyposaline SP- Cl contained water that was dominated by Cl^- anions, SO_4^{2-} concentrations were also high relative to Cl^- , and therefore this lake appeared below the origin in the ordination diagram. The relative influence of Ca^{2+} can be observed by the proximity of the sites to the Ca^{2+} vector in the site-environment biplot (Figure 2-5B). Surface area contributed to the formation of the first axis because the study lakes containing water dominated by $\text{SO}_4^{2-}/\text{CO}_3^{2-}$ were larger than the study lakes with Cl^- -dominated water (Table 2-2).

2.4 Discussion

There was considerable variation in water chemistry among all of the study lakes. Each of the subsaline study lakes had slightly different chemical characteristics, but GW-D, WR-D, and BP-D were most similar in terms of salinity and nutrients. The large differences in SO_4^{2-} concentrations (0.6 to 649 mg/l) observed among subsaline lakes were likely related to the amount of gypsum ($\text{CaSO}_4 \cdot \text{H}_2\text{O}$) that was in the bedrock underlying the lakes (Moser et al. 1998). GL-D differed from all other study lakes because it was the deepest and the only oligotrophic lake studied, as well as being the only subsaline lake that was dominated by SO_4^{2-} anions. Although phosphorus is released from lake sediments at higher SO_4^{2-} concentrations (Caraco et al. 1989), TP concentrations were low (14.7 $\mu\text{g/l}$). This nutrient was likely precipitated as calcium

phosphate (CaPO_4) (Wetzel 1983) since Ca^{2+} is provided in abundance by the limestone bedrock (Lemmen et al. 1994). FP-D was unique among the study lakes because it shared chemical characteristics with both the subsaline and the Cl^- -dominated saline lake water. Although FP-D had surface waters that remained subsaline for most of the sampling period, the water column of this lake was inversely stratified by the end of the summer when the bottom stratum was saline.

The saline study lakes in northern Alberta, which contain water dominated by Cl^- anions, are not representative of lake water in the predominantly freshwater boreal region in which they are found (Moser et al. 1998). With the exception of one saline lake with water dominated by CO_3^{2-} anions that has been reported in the subarctic (Pienitz et al. 1992), most lakes north of the prairies and aspen parklands contain fresh water (Pienitz et al. 1997a, Moser et al. 1998, Rühland and Smol 1998) except for those within close proximity of the sea (Pienitz et al. 1997b). The salinity of the study lakes in northern Alberta is a result of groundwater discharging high concentrations of NaCl salt (Camsell 1917, Tsui 1982). Other isolated examples of saline lakes derived from NaCl springs are found in the Saskatchewan River Delta (Hammer 1993, Hammer et al. 1975) and on the Interior Plateau of British Columbia (Wilson et al. 1994). The chloride-dominated waters that I studied had low total alkalinities ($<305 \text{ mg/l}$), and the reduced ratio of sulphate to chloride at higher salinities may have been a result of $\text{CaSO}_4 \bullet \text{H}_2\text{O}$ precipitation (Evans and Prepas 1996). However, SO_4^{2-} was elevated in SP-Cl relative to the other lakes with Cl^- -dominated water and this may be related to spatial variation in the amount of gypsum in bedrock underlying the lakes. The relatively low TP concentrations in the lake water (18 to $85 \text{ } \mu\text{g/l}$) likely resulted from precipitation of phosphorus as

CaPO_4 (Wetzel 1983). Although TN was high (741 to 1673 $\mu\text{g/l}$), amounts of biologically available NO_2^- and NO_3^- were low (2 to 39 $\mu\text{g/l}$) and Moser et al. (1998) reported that nitrogen deficiencies are common among WBNP freshwater lakes. However, nitrogen fixation in benthic algal mats, such as those found in GB-Cl and SP-Cl, may contribute significantly to the amount of TN in saline lakes (Horre and Galat 1985, Jellison et al. 1993). The presence of halite deposits in combination with local and regional groundwater flow regimes were likely dominant forces in determining the salinity of surface water (Toth 1999) that I studied in northern Alberta.

In contrast to Cl^- -dominated saline lake water that is infrequently encountered in North America, the three study lakes sampled in central Alberta are similar to many of the saline lakes on the northern Great Plains of Canada (e.g. Rawson and Moore 1944, Bierhuizen and Prepas 1985, Hammer 1986b, Last 1992) and the United States of America (e.g. Winter 1977, Gorham et al. 1983, Last 1992, Blinn 1993). The high concentrations of SO_4^{2-} and $\text{HCO}_3^-/\text{CO}_3^{2-}$ in these lakes, which occur in excess of 3000 mg/l , have been attributed to weathering of Cretaceous black shale bedrock in combination with evaporative processes (Last and Schweyen 1983, Blinn 1993). TP concentrations are in the eutrophic range (16 to 386 $\mu\text{g/l}$) for many freshwater lakes in central Alberta (Prepas and Trew 1983). Saline lakes in this region can be hypereutrophic with concentrations >1000 $\mu\text{g/l}$ TP as a result of evaporative concentration (Campbell and Prepas 1986) and phosphorus release from bottom sediments at high SO_4^{2-} concentrations (Caraco et al. 1989). However, despite the elevated TP concentrations, algal productivity can be Fe^{2+} limited in these SO_4^{2-} -dominated hyposaline lakes (Evans and Prepas 1997). Unlike the $\text{SO}_4^{2-}/\text{CO}_3^{2-}$ -dominated

lake water that I studied, low phosphorus availability has been reported for Saskatchewan lakes and appears to be influenced by the interaction of high DOC, pH and ionic composition (Waiser and Robarts 1995). Studies of other highly alkaline saline lakes have found that bacterial oxidation of NH_3 maintains pools of biologically available NO_2^- (Joye et al. 1999). Climate, topographic position within the drainage system, and groundwater flow regimes govern salt concentration and ionic composition of lake water on the northern Great Plains (Covich et al. 1997).

Although the number of lakes sampled in this study was small, zooplankton diversity was reduced at high salinities compared to low salt concentrations, which supports the findings of many other studies (e.g. Williams et al. 1990, Hammer 1990, Frey 1993, Hammer 1993, Williams 1993). Zooplankton have substantial spatial (e.g. Pinel-Alloul et al. 1995) and temporal (e.g. Arnott et al. 1998) variability, and my sampling design captures only some of these trends. The use of a smaller-meshed net (48 μm) would have caught rotifer species $<64 \mu\text{m}$ that were not considered in this study (Stemberger and Lazorchak 1994). Several sampling stations rather than one central sampling point may have provided better estimates of rare species, particularly in the subsaline lakes where diversity was higher. However, single stations in small lakes, which was the case for this study, have been found to yield a good representation of the relative abundance of dominant species and capture at least 80% of total species present (Patalas and Salki 1993). Although samples were collected during daylight, diurnal migration of zooplankton was accounted for by sampling throughout the water column. However, it is possible that zooplankton hiding in the sediments could have been missed. Different species assemblages can occur within the same lake between different years

(Arnott et al. 1999), and this is supported by slight differences in community composition between 1984 and 1999 June samples for the --SO_4 study lakes. For example, *Daphnia similis* was absent from PN- SO_4 and FL- SO_4 in June of 1999, but present during the same month in 1984. *Brachionus urceolaris* was abundant in these lakes in 1999, but absent in 1984.

Lake surface area, mean depth and the presence of associated streams were variable among the study lakes, and likely affected the diversity and composition of zooplankton that were observed. Deeper, larger lakes tend to be characterized by greater zooplankton species richness and abundance than smaller, shallower lakes where there is higher predation pressure by fish and invertebrates (Keller and Conlin 1994). With the exception of deepest GL-D, the saline lakes with $\text{SO}_4^{2-}/\text{CO}_3^{2-}$ -dominated water had the largest surface areas and the deepest mean depths (Table 2-1), but an absence of inflowing or outflowing streams (Campbell and Prepas 1986) likely restricted opportunities for fish to enter these lakes. In contrast, the saline lakes containing Cl^- -dominated water were smaller and shallower (Table 2-1), but had surface connections to other rivers and lakes. Nine-spined stickleback (*Pungitius pungitius*) were abundant in these shallow but well connected systems. Predation of zooplankton by both nine-spined stickleback (Nelson and Paetz 1992) and corixids (Scudder 1983) was likely strong in the saline lakes containing Cl^- -dominated water compared to the lakes with isolated basins containing water dominated by $\text{SO}_4^{2-}/\text{CO}_3^{2-}$.

Almost all rotifer and crustacean zooplankton species were most abundant in the subsaline study lakes. Species such as *Lophocharis salpina*, *Keratella quadrata*, and *Notholca acuminata* that ordinated with the Cl^- vector in the species-environment

ordination biplot for the subsaline lakes also appeared in low densities in the hyposaline saline lake water dominated by Cl^- . In contrast to ordinations of the saline lakes, the genus *Hexarthra* was not associated with the Cl^- vector and may have been a different species in the subsaline lakes than what was observed in the saline lakes. *Lecane luna* was the only species that was closely affiliated with elevated Mg^{2+} concentrations, but pH influenced many species. Rotifers such as *Lecane ohioensis*, *Brachionus quadridentatus*, *Synchaeta* sp., *Mytilina ventralis* var. *brevispina*, *Polyarthra vulgaris* were most strongly correlated with elevated pH, and reached highest densities at a pH of 9.2 in BP-D.

Some zooplankton species that were abundant in the hypo- and meso-saline lakes appeared in low densities in the subsaline lakes. Although environment-species relationships were not detectable for crustacean zooplankton in the subsaline lakes, *Leptodiatomus sicilis* was not abundant but strongly associated with SO_4^{2-} in GL-D. The halophile *Brachionus plicatilis* was most abundant in FP-D (Table 2-4) at the end of June when the lake underwent a rapid change from hyposaline to subsaline. Water chemistry was analyzed from only surface strata, and saline waters at the bottom of FP-D are not reflected in Figure 2-5B. *B. plicatilis* was periodically present but not abundant on several sampling occasions in FP-D, which may have resulted from the presence of saline water near the bottom of the lake (Figure 2-2) rather than subsaline conditions in surface waters as is suggested by Figure 2-5A. The subsaline lakes were inhabited by species with a mixture of salinity tolerances (Tables 2-4 and 2-5).

Zooplankton communities in the Cl^- -dominated saline lake water of northern Alberta most closely resembled descriptions of Cl^- -dominated waters in arid regions of the Western United States (Hammer 1986a, Wurtsbaugh and Berry 1990). Lakes with

Cl⁻-dominated waters in northern Alberta were characterized by an abundance of *Brachionus plicatilis*, and species of *Hexarthra* and *Cletocamptus* that most likely were *H. fennica* (Levander) and *C. albuquerquensis* (Herrick) (Hammer 1993). Hypersaline Great Salt Lake, Utah originated approximately 15 000 years ago and is usually only inhabited by brine shrimp, *Artemia franciscana*, which tends to be found in highly saline waters irrespective of ion composition (e.g. Ivanova 1991, Hammer 1993, Bos et al. 1996, Williams 1998). The south basin of Great Salt Lake experienced unusually low salinities (50 g/l compared to 250 g/l in the mixolimnion) from 1985 to 1987, and had concentrations of nutrients that were similar to the study lakes in Alberta. Under the mesosaline conditions from 1985 to 1987 in Great Salt Lake, *Brachionus plicatilis* became the most abundant zooplankter with a peak density of 745 000 individuals/l. Other less abundant zooplankton included *Cletocamptus albuquerquensis* and *Diaptomus connexus*, as well as the predatory corixid, *Trichocorixa verticalis* that contributed to the depletion of *Artemia franciscana* (Wurtsbaugh and Berry 1990). Most North American salt lakes appeared post-glaciation 10 000 years ago and have less diverse zooplankton communities with narrower salinity tolerances (Hammer 1993) than the ancient saline lakes that contain Cl⁻-dominated water in Australia, Africa, (Frey 1993), and South America (Bayly 1993).

Halophilic zooplankton communities in Ca²⁺-rich saline lake water dominated by Cl⁻ anions differed from those found in alkaline, hypereutrophic SO₄²⁻/CO₃²⁻-dominated lake water. Most of the zooplankton that were observed in the SO₄²⁻/CO₃²⁻-dominated saline lake water consisted of large crustaceans. *Leptodiaptomus sicilis*, *Diaptomus nevadensis* and *Daphnia similis* are crustacean zooplankton that are characteristic of

SO₄²⁻-dominated saline waters in North America (e.g. Walker 1975, Reynolds 1979, Galat and Robinson 1983, Hammer 1993, Evans et al. 1995, Leland and Berkas 1998) and were prevalent in the central Alberta study lakes. Bos et al. (1996) reported that among 111 lakes dominated by SO₄²⁻ and CO₃²⁻/HCO₃²⁻ in the interior of British Columbia, halophilic calanoid copepods were also associated with lower Mg²⁺ and Ca²⁺ concentrations in lake water. In SO₄²⁻-dominated saline waters in Siberia, the calanoid copepod *Arctodiaptomus salinus* was the most abundant zooplankter in a hyposaline, fishless lake (Zotina et al. 1999). The only rotifer species that occurred in high abundance in the SO₄²⁻/CO₃²⁻ saline study lakes in this study was *Brachionus urceolaris*, which has also been recorded in hyposaline SO₄²⁻-dominated waters of Devils Lake, North Dakota (Leland and Berkas 1998).

Rotifers that were abundant in the Cl⁻-dominated saline lake water have been reported by other studies to also occur in high densities in SO₄²⁻/CO₃²⁻-dominated lake water. *Brachionus plicatilis* and *Hexarthra fennica* have been reported to be tolerant of waters dominated by different anions over a wide range of salinities (300 to greater than 250 000 mg/l) (Hammer et al. 1975, Hammer 1986a, Hammer 1993). However, there is no record of *L. sicilis*, *D. nevadensis*, and *D. similis* in Cl⁻-dominated waters in North America (e.g. Wurtsbaugh and Berry 1990). Hammer (1993) indicated that *L. sicilis*, *D. nevadensis*, and *D. similis* belonged to entirely different lake categories from *B. plicatilis* and *A. franciscana* based on salinity gradient, pH, Secchi depth, and mean depth. However, Hammer (1993) sampled across lakes with variable ion composition and the role of different ions on zooplankton communities was unclear. Cl⁻-dominated waters are less stressful for osmoregulation than SO₄²⁻-dominated waters for fish larvae (Koel and

Peterka 1995), and these differences in ion strength may also reflect variation in selective pressure on zooplankton that inhabit salt lakes.

It is possible that taxa such as *L. sicilis*, *D. nevadensis*, and *D. similis* have adapted to conditions of water chemistry found only in $\text{SO}_4^{2-}/\text{CO}_3^{2-}$ -dominated saline waters, which are characteristic of most natural salt lakes in North America.

Alternatively, predation by corixids (e.g. Scudder 1983) and stickleback fish (Nelson and Paetz 1992) may have diminished crustacean zooplankton in the Cl^- -dominated saline lakes that would have otherwise been abundant under fishless conditions such as in the study lakes containing $\text{SO}_4^{2-}/\text{CO}_3^{2-}$ -dominated water. However, *L. sicilis* was found co-occurring in low densities with nine-spined stickleback in the SO_4^{2-} -dominated, oligotrophic, subsaline GL-D study lake. Additionally, fish were not detected in HC-Cl and these Cl^- -dominated waters contained largely a monoculture of a single rotifer species (*Brachionus plicatilis*). Further, *L. sicilis* was reported by Leland and Berkas (1998) to be abundant in hyposaline Devils Lake, North Dakota, which contains both SO_4^{2-} -dominated water and planktivorous fish. *L. sicilis*, therefore, appears to be associated with SO_4^{2-} . However, contrasting water chemistry and different predation regimes likely both contributed to the distinct zooplankton communities that I observed among these saline lakes.

Studies involving a larger set of saline lakes and experimentally manipulated mesocosms would assist in teasing out the effects of ions, nutrients and fish on zooplankton communities in saline lakes. Inland waters dominated by Cl^- are abundant the continents of Australia and Africa, but these landscapes are older and have different aquatic communities than in North America (Hammer 1986a). Differences in

zooplankton communities between lake water with Cl^- and $\text{SO}_4^{2-}/\text{CO}_3^{2-}$ -dominated salts have not been studied previously, and are important when considering the effects of salt pollution on surface water.

Table 2-1. The location and morphometry of saline and subsaline study lakes. At the end of the lake designations, -SO₄ refers to the SO₄²⁻/CO₃²⁻-dominated saline waters in central Alberta, and -Cl and -D respectively refer to Cl⁻-dominated saline waters and subsaline waters found in the northern part of the province.

Saline Lakes	Location	Surface Area (ha)	Maximum Depth (m)	Mean Depth (m)
OL-SO ₄	53°05'N, 111°36'W	52	1.7	1.3
PN-SO ₄	52° 52'N, 111°29'W	139	3.1	2.1
FL-SO ₄	52° 50'N, 111°19'W	32	2.1	1.4
GB-Cl	59° 25'N, 111°30'W	38	0.5	0.3
HC-Cl	59° 31'N, 111°27'W	4.4	0.5	0.3
SP-Cl	59° 48'N, 112°01'W	32	0.7	0.3
SL-Cl	57° 04'N, 111°31'W	150	1	0.5
Subsaline Lakes				
GL-D	60° 00'N, 112°37'W	15	11	3.4
GW-D	59° 31'N, 111°27'W	1.7	2.2	1.0
BP-D	59° 31'N, 111°27'W	0.78	2.4	0.8
FP-D	59° 31'N, 111°27'W	3.3	1.7	0.8
WR-D	59° 31'N, 111°27'W	4.1	2.5	1.3

Table 2-2. Measurements of TDS (mg/l), conductivity (cond., $\mu\text{S}/\text{cm}$), major nutrient ($\mu\text{g}/\text{l}$) and ion (mg/l) concentrations for the study lakes over summer of 1999. pH, DOC (mg/l), turbidity (NTU), colour (mg/l Pt), and chl *a* ($\mu\text{g}/\text{l}$) are also presented. SO_4^{2-} -dominated saline waters in central Alberta ($-\text{SO}_4$) were measured in only June. Averages for June, July, August and September were calculated for Cl^- -dominated saline waters ($-\text{Cl}$) and subsaline waters ($-\text{D}$), with the exception of SL-Cl that was sampled only in June and August. NO_2 and NO_3 ($\mu\text{g}/\text{l}$) were measured in July for most of the lakes, only once in August for SL-Cl, and n.d. indicates where no data was available. Salinity categories are given according to Hammer (1986a): hypersaline is $>50\,000$ mg/l TDS, mesosaline is 20 to 50 000 mg/l TDS, and hyposaline is 3 to 20 000 mg/l TDS.

Saline Lakes	OL- SO_4	PN- SO_4	FL- SO_4	GB-Cl	HC-Cl	SP-Cl	SL-Cl
Salinity Category	Hyper-Saline	Hypo-Saline	Hypo-Saline	Meso-Saline	Meso-Saline	Hypo-Saline	Hypo-Saline
TDS	96 228.5	14 277.0	8 028.5	26 318.4	25 605.5	12 675.9	7282.0
Cond.	73 336	16 441	10 230	40 217	36 805	18 926	12 144
TP	25 530.0	2 915.0	1 322.0	18.0	64.2	29.2	84.8
TN	862.2	47.2	61.6	741.2	847.7	1673.2	1472.9
NO_2+NO_3	n.d.	n.d.	n.d.	2.2	39.1	1.8	10.0
Na^+	37 048.0	4 898.0	2 857.0	9519.8	8205.2	3726.0	2491.5
Ca^{2+}	0.63	4.0	2.8	457.4	679.0	805.7	145.9
K^+	606.8	127.8	79.3	7.2	24.8	4.3	7.2
Mg^{2+}	45.6	94.7	58.3	35.7	235.8	48.1	64.0
Fe^{2+}	2.0	0.2	1.2	0.13	0.15	0.06	0.15
Mn^{3+}	0.1	0.02	0.03	0.04	0.06	0.04	0.1
Cl^-	856.0	280.0	170.7	16 764.7	13 485.4	5672.2	3832.2
SO_4^{2-}	3 387.1	7 544.0	3 590.0	1209.2	1411.6	2219.1	339.9
CaCO_3	45 232.0	3 264.0	3 778.4	58.8	105.5	54.1	137.2
HCO_3^-	9 377.0	2 666.0	3 024.0	71.7	128.6	65.9	167.4
CO_3^{2-}	22 508.0	646.0	778.0	0	0	0	0
PH	10.2	9.6	9.3	8.4	8.8	8.2	8.3
DOC	290.1	71.8	75.2	13.9	17.4	29.2	20.4
Turbidity	2.6	10.0	27.0	1.4	3.4	3.3	2
Colour	67.3	35.0	96.7	10.6	67.6	17.0	33.6
Chl <i>a</i>	2.4	4.9	2.7	3.2	12.8	6.0	2.6
Subsaline Lakes	GL-D	GW-D	BP-D	FP-D	WR-D		
TDS	1089.8	981.9	608.4	840.3	556.0		
Cond.	1267	1805	862	1222	824		
TP	14.7	65.3	38.6	124.5	31.5		
TN	620.1	1150.3	1046.6	1520.0	908.2		
NO_2+NO_3	47.3	37.5	26.1	32.7	18.7		
Na^+	30.7	225.4	68.5	127.2	73.5		
Ca^{2+}	193.1	77.0	50.5	70.8	39.2		
K^+	2.8	8.0	4.9	3.6	6.0		
Mg^{2+}	47.2	43.0	42.5	39.2	32.4		
Fe^{2+}	0.003	0.3	0.02	0.4	0.1		
Mn^{3+}	0.007	0.2	0.007	0.05	0.005		
Cl^-	29.4	465.2	133.4	246.8	178.0		
SO_4^{2-}	649.0	16.2	90.2	45.1	0.6		
CaCO_3	83.1	130.0	170.0	203.0	133.6		
HCO_3^-	101.3	157.0	1046.6	239.3	161.3		
CO_3^{2-}	0	0.7	3.3	4.0	0.8		
PH	8.1	7.8	8.6	7.9	7.8		
DOC	10.5	26.8	24.4	38.7	22.1		
Turbidity	0.7	2.2	0.9	6.6	3.2		
Colour	9.6	93.9	77.7	205.9	92.5		
Chl <i>a</i>	4.0	4.1	6.9	25.3	3.6		

Table 2-3. Salinity categories (total dissolved solids (TDS), Hammer 1986a), and summer measurements of conductivity (cond., $\mu\text{S}/\text{cm}$), total phosphorus (TP, $\mu\text{g}/\text{l}$), ions (mg/l), pH, and chl *a* ($\mu\text{g}/\text{l}$) for the saline study lakes located in central Alberta. Values are averaged for June, July and August from 1983 to 1992 for PN-SO₄ and FL-SO₄ (Evans 1995) and from 1983 to 1984 for OL-SO₄ (Bierhuizen and Prepas 1985).

	OL-SO₄	PN-SO₄	FL-SO₄
Salinity Category	Hypersaline >50 000 mg/l TDS	Hyposaline 3 to 20 000 mg/l TDS	Hyposaline 3 to 20 000 mg/l TDS
Cond.	53 532	14 547	11 196
TP	14 232	3 410	2 116
Na⁺	23 241	3 601	2 783
Ca²⁺	433	113	74
K⁺	19	14	14
Mg²⁺	108	96	55
Cl⁻	521	181	155
SO₄²⁻	29 422	4 693	2 905
Alk	24 329	2 815	2 944
pH	9.9	9.3	9.3
Chl <i>a</i>	5.0	6.7	3.9

Table 2-4. Peak density observed for rotifer species (# individuals/l lake water) in each category of lake-water salinity (Hammer 1986a) over the summer of 1999. Dominant anions for each lake category are indicated in brackets.

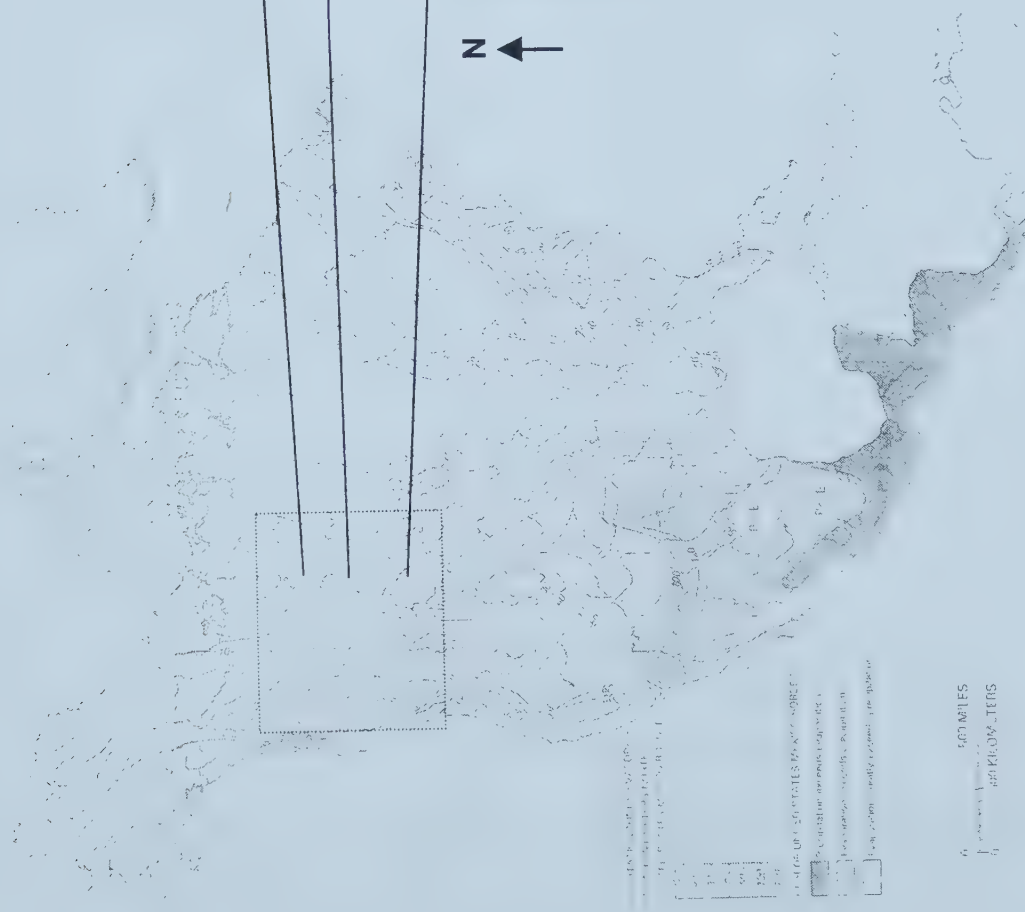
Species	Hyper-Saline (CO ₃ /SO ₄)	Hypo-Saline CO ₃ /SO ₄	Meso-Saline Cl	Hypo-Saline Cl	Sub-Saline SO ₄ or Cl
<i>Ascomorpha ecaudis</i>	0	0	0	0	89.2
<i>Ascomorpha ovalis</i>	0	0	0	0	112.7
<i>Anuraeopsis fissa</i>	0	0	0	0	25.5
<i>Asplanchna brightwelli</i>	0	0	0	0	14.6
<i>Asplanchna priodonta</i>	0	0	0	0	427.8
<i>Brachionus plicatilis</i>	0	0	869.4	202.2	2712.0*
<i>Brachionus quadridentatus</i>	0	0	0	1.1	18.1
<i>Brachionus rubens</i>	0	0	0	0.37	0
<i>Brachionus urceolaris</i>	0.02	26.3	0	0	0
<i>Collotheca mutabilis</i>	0	0	0	0	171.1
<i>Collotheca pelagica</i>	0	0	0	0	2.1
<i>Colurella obtusa</i>	0	0	0	0	6.4
<i>Colurella uncinata</i>	0	0	0	0	7.5
<i>Encentrum</i> sp.	0	0	0	0	1.1
<i>Filinia longiseta</i>	0	0	0	0	2383.0
<i>Gastropus stylifer</i>	0	0	0	0	113.1
<i>Hexarthra</i> sp.	1.8	0.08	0.2	752.4	3.0
<i>Keratella cochlearis</i>	0	0.3	0.03	0	1440.6
<i>Keratella hiemalis</i>	0	0	0	1.4	122.1
<i>Keratella quadrata</i>	0.02	0.1	2.7	19.0	6732.5
<i>Keratella serrulata</i>	0	0	0	0	29.3
<i>Keratella testudo</i>	0	0.1	0	0	3371.9
<i>Keratella ticinensis</i>	0	0	0	0	0.63
<i>Keratella valga</i>	0	0	0	0	1.1
<i>Lecane luna</i>	0	0	0	0	12.7
<i>Lecane ohioensis</i>	0	0	0	0	6.0
<i>Lepadella acuminata</i>	0	0	0	0	9.0
<i>Lepadella patella</i>	0	0	0	0	12.7
<i>Lophocharis salpina</i>	0	0	0	1.6	3.0
<i>Monostyla bulla</i>	0	0	0	0	239.1
<i>Monostyla closterocerca</i>	0	0	0	0	12.7
<i>Monostyla lunaris</i>	0	0	0	0	12.7
<i>Monostyla quadridentus</i>	0	0	0	0	6.4
<i>Mytilina ventralis</i> var <i>brevispina</i>	0	0	0	0	52.7
<i>Notholca acuminata</i>	0	0	0	5.1	25.5
<i>Notommata</i> sp.	0	0	0	0	51.0
<i>Platytias patulus</i>	0	0	0	0	29.3
<i>Polyarthra dolichoptera</i>	0	0	0	0	12.7
<i>Polyarthra vulgaris</i>	0	0.4	0	0	3130.4
<i>Pompholyx</i> sp.	0	0	0	0	732.6
<i>Synchaeta</i> sp.	0	0	0	0	3975.0
<i>Testudinella patina</i>	0	0	0	0	11.7
<i>Trichocerca longiseta</i>	0	0	0	0	7.5
<i>Trichocerca lophoessa</i>	0	0	0	0	3.0
<i>Trichocerca multirinis</i>	0	0	0	0	167.2
<i>Trichocerca rattus</i>	0	0	0	0	4.9
<i>Trichotria pocillum</i>	0	0	0	0	9.0
<i>Trichotria tetractis</i>	0	0	0	0	6.4
<i>Vanoyella globosa</i>	0	0	0	0	3.0

Table 2-5. Peak density observed for crustacean species (# individuals/l lake water) in each category of lake-water salinity (Hammer 1986a) over the summer of 1999. Dominant anions for each lake category are indicated in brackets.

Species	Hyper-saline (CO ₃ /SO ₄)	Hypo-Saline (CO ₃ /SO ₄)	Meso-saline (NaCl)	Hypo-saline (NaCl)	Sub-Saline (Cl or SO ₄)
Anostracans					
<i>Artemia franciscana</i>	27.9	0	0	0	0
Calanoid Copepods					
<i>Agalodiaptomus leptopus</i>	0	0	0	0	68.3
<i>Diaptomus arcticus</i>	0	0	0	0	12.7
<i>Diaptomus nevadensis</i>	0	0.4	0	0	0
<i>Leptodiaptomus nudus</i>	0	0	0	17.6	0
<i>Leptodiaptomus sicilis</i>	0	5.9	0	0	0.2
Cyclopoid Copepods					
<i>Acanthocyclops carolinianus</i>	0	0	0	0.80	0
<i>Acanthocyclops robustus</i>	0	0	0	0	14.6
<i>Acanthocyclops venustoide</i>	0	0	0	0	97.1
<i>Acanthocyclops vernalis</i>	0	0	0	0	4.9
<i>Diacyclops navus</i>	0	0	0	0.5	3.2
Harpacticoid Copepods					
<i>Cletocamptus</i> sp.	0	0	61.1	0.80	0
Cladocerans					
<i>Alona circumfimbriata</i>	0	0	0	0	6.4
<i>Alona costata</i>	0	0	0	0	3.0
<i>Alona guttata</i>	0	0	0	0	4.9
<i>Alona rectangula</i>	0	0	0	0.2	2.4
<i>Bosmina</i> sp.	0	0	0.03	0	27.9
<i>Ceriodaphnia laticaudata</i>	0	0	0	0	76.4
<i>Ceriodaphnia pulchella</i>	0	0	0	3.7	0
<i>Chydorus brevilabris</i>	0	0	0	0	95.5
<i>Chydorus piger</i>	0	0	0	0	19.1
<i>Chydorus sphaericus</i>	0	0	0	0	19.1
<i>Daphnia parvula</i>	0	0	0.03	0	38.2
<i>Daphnia pulicaria/pulex</i>	0	0	0	6.4	262.3
<i>Daphnia rosea</i>	0	0	0	0	29.3
<i>Daphnia schoedleri</i>	0	0	0	0	31.8
<i>Polyphemus pediculus</i>	0	0	0	0	6.4

Table 2-6. List of zooplankton species present in saline lakes containing Cl⁻-dominated waters, but that were not included in the CCA of all saline study lakes because of the restricted number of species that could be included in the analysis. *Brachionus quadridentatus*, *Keratella quadrata*, and *Daphnia parvula* were included in the CCA, and were positively correlated with the following list of taxa that were not included in the CCA.

Zooplankton Species in CCA	Correlated Zooplankton Taxa	<i>r</i>
<i>Brachionus quadridentatus</i>	<i>Brachionus rubens</i>	0.60
	<i>Keratella hiemalis</i>	0.50
	<i>Notholca acuminata</i>	0.55
	<i>Leptodiptomus nudus</i>	0.80
	<i>Acanthocyclops carolinianus</i>	0.55
	<i>Diacyclops navus</i>	0.80
	<i>Daphnia pulicaria/pulex</i>	0.50
<i>Keratella quadrata</i>	<i>Keratella hiemalis</i>	0.43
	<i>Keratella testudo</i>	0.44
	<i>Notholca acuminata</i>	0.42
	<i>Polyarthra vulgaris</i>	0.44
	<i>Daphnia pulicaria/pulex</i>	0.43
<i>Daphnia parvula</i>	<i>Bosmina</i> sp.	1.0



From: Winter, T.C., U.S. Geological Survey. 1989.

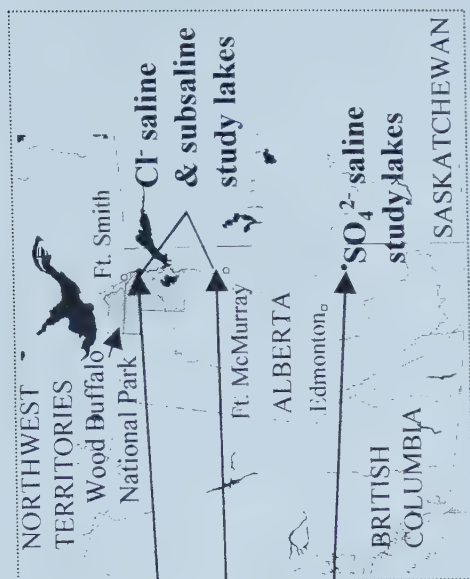


Figure 2-1. The location of study lakes in Wood Buffalo National Park and Alberta, Canada in relation to the distribution of the difference between precipitation and open water evaporation in North America.

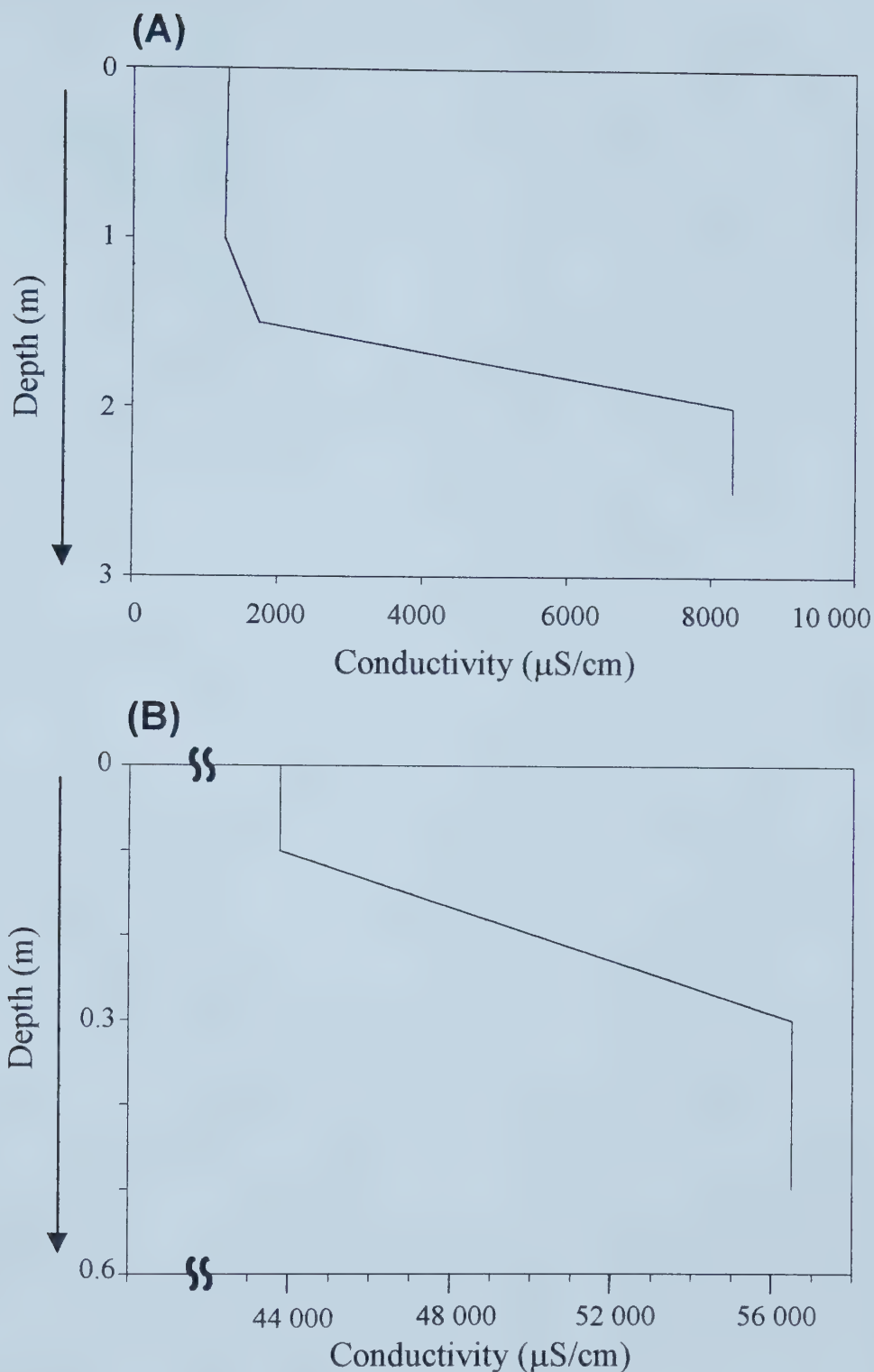


Figure 2-2. Depth profiles of conductivity ($\mu\text{S}/\text{cm}$) indicating inverse stratification of water in (A) subsaline FP-D and in (B) mesosaline HC-Cl. This phenomenon was displayed in September in FP-D and throughout the summer in HC-Cl. Surface areas of FP-D and HC-Cl were 3.3 and 4.4 ha, respectively.

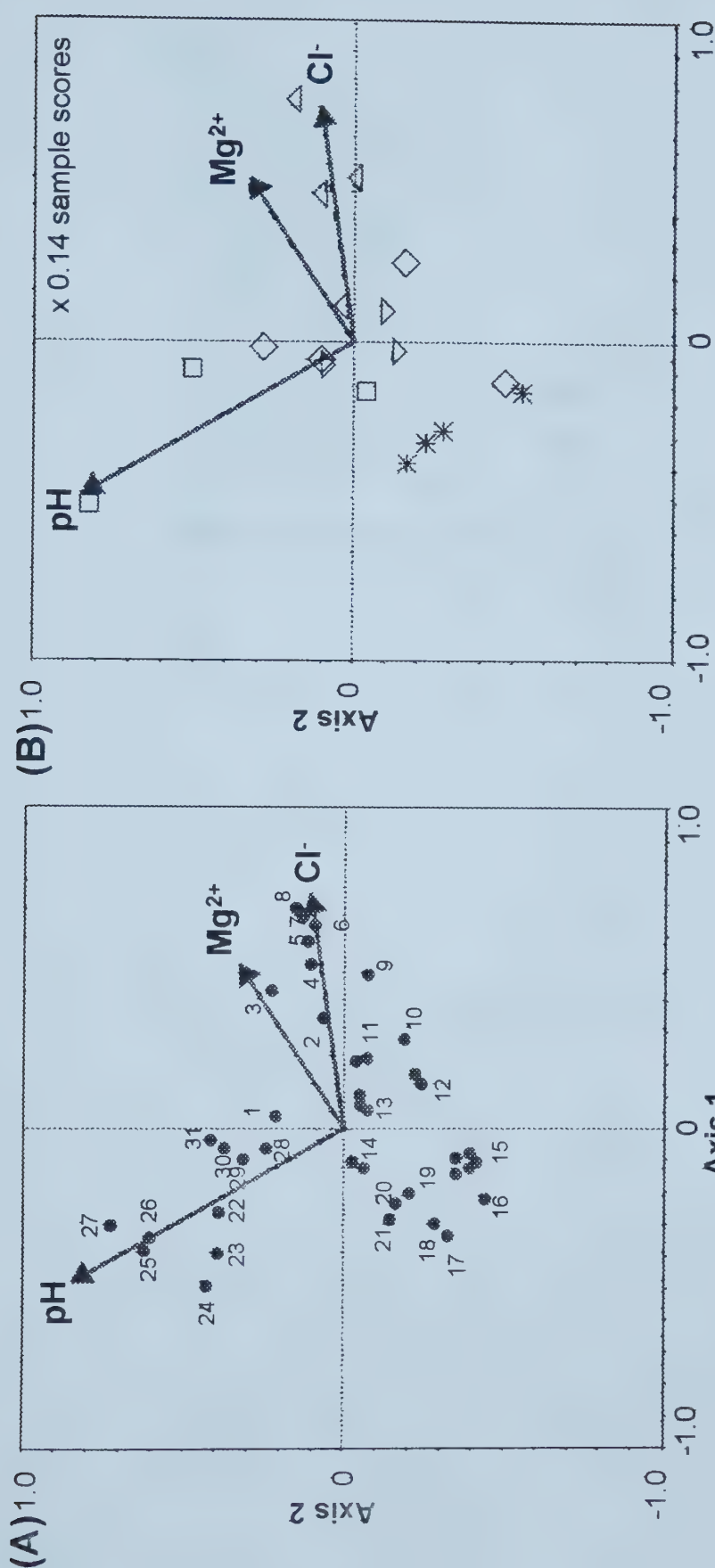


Figure 2-3. Ordination biplots of (A) species and environmental variables, and (B) sites and environmental variables from partial RDA of rotifer species in the subsaline study lakes. In (A), species ordination positions are represented by numbers: 1. *Testudinella patina*, 2. *Colurella obtusa*, 3. *Lecane luna*, 4. *Monostyla quadridentatus*, 5. *Notoommata* sp., 6. *Notholca acuminata*, 7. *Keratella quadrata* and *Trichotria tetractis*, 8. *Lepadella patella*, 9. *Lophocharis salpina*, 10. *Anuraeopsis fissa*, 11. *Asplanchna brighthwelli*, 12. *Monostyla clostercera* and *Monostyla lunaris*, 13. *Asplanchna priodonta*, 14. *Colurella valga*, 15. *Brachionus plicatilis*, 16. *Lepadella acuminata*, 17. *Keratella cochlearis*, 18. *Gastropus stylifer* and *Trichocerca lophoessa*, 19. *Vanoyella globosa*, 20. *Trichocerca multirinis*, 21. *Ascomorpha ovalis*, 22. *Mytilina ventralis* var. *brevispina*, 23. *Platylas platylas*, 24. *Polyarthra vulgaris*, 25. *Lecane ohioensis*, 26. *Brachionus quadridentatus*, 27. *Synchaeta* sp., 28. *Polyarthra dolichoptera*, 29. *Keratella serrulata*, 30. *Colurella uncinata* and *Trichocerca longiseta*, 31. *Monostyla bulla*. In (B), the symbols represent samples from each of the lakes: squares = BP-D, diamonds = FP-D, upward triangles = GL-D, upside-down triangles = GW-D, and stars = WR-D.

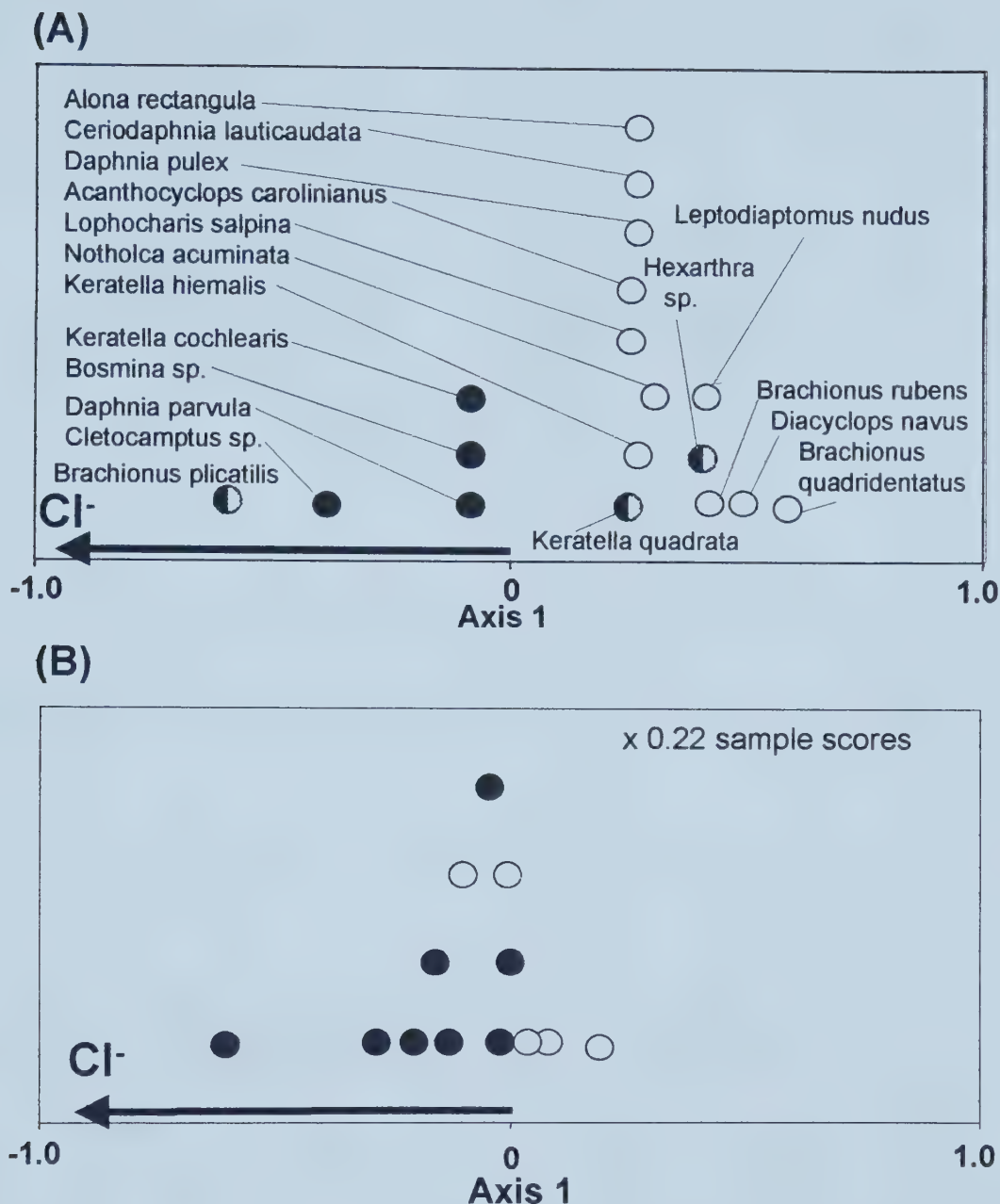


Figure 2-4. Ordination biplots of (A) species and environmental variables, and (B) sites and environmental variables from partial RDA of rotifer and crustacean zooplankton species within the saline lakes with Cl^- -dominated water. In (B), the filled symbols represent samples from the mesosaline lakes and empty symbols represent the hyposaline lakes. In (A), filled symbols indicate species found only in the mesosaline lakes, empty symbols are species found only in the hyposaline lakes, and black-and-white symbols are species that appeared in both the mesosaline and hyposaline study lakes.

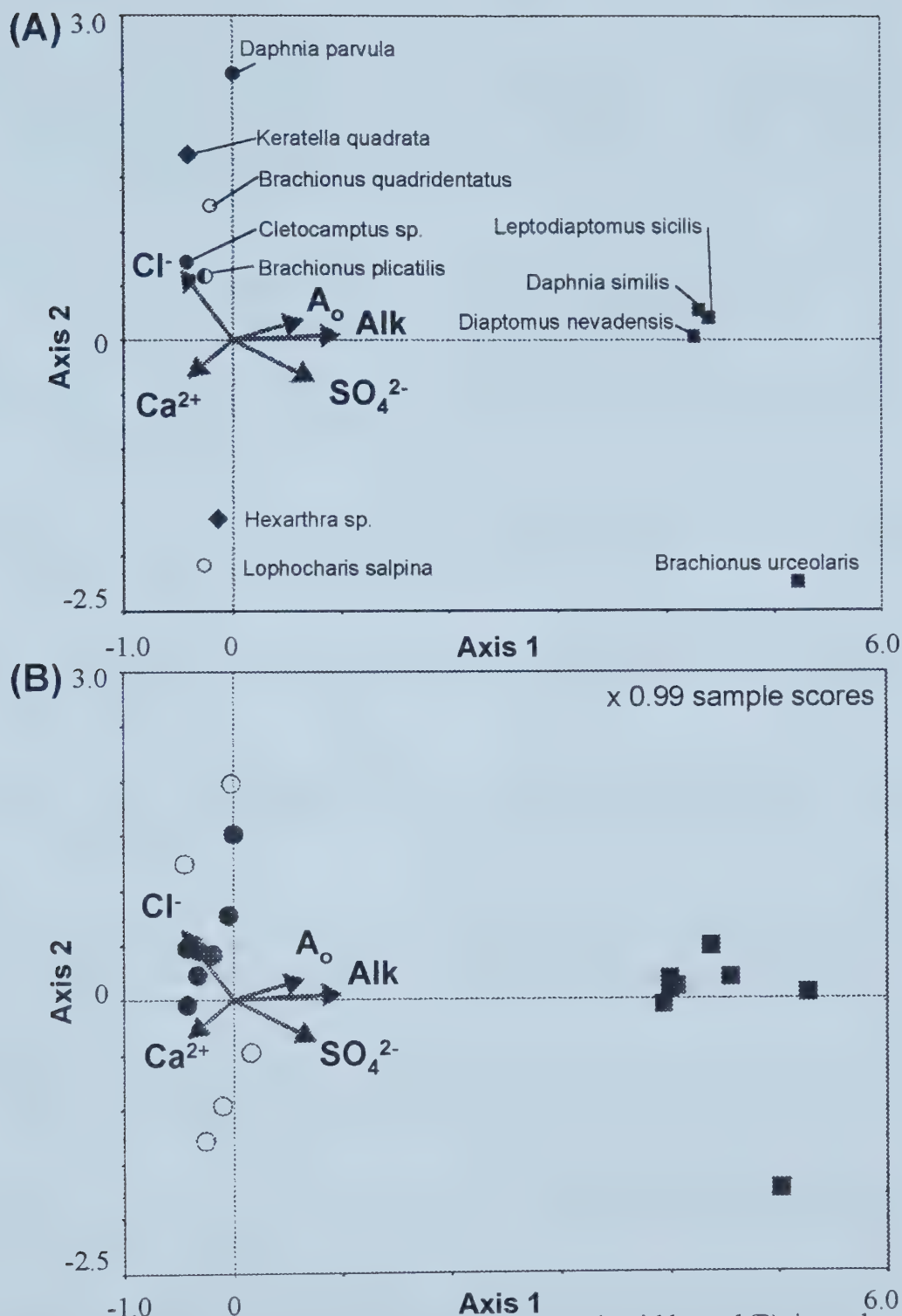


Figure 2-5. Ordination biplots of (A) species and environmental variables, and (B) sites and environmental variables from partial CCA of rotifer and crustacean zooplankton found in Cl^- -dominated (circles) and $\text{SO}_4^{2-}/\text{CO}_3^{2-}$ -dominated (squares) saline lake water. Of the Cl^- -dominated waters in (A), species found at hyposaline concentrations are represented by empty circles, species found at mesosaline concentrations are solid circles, and species found at both meso- and hyposaline concentrations are black-and-white circles. Species found only in $\text{SO}_4^{2-}/\text{CO}_3^{2-}$ saline lake water are represented by solid squares in (A). Zooplankton species that appeared in both lakewater ion types are indicated by solid diamonds. Abbreviations: A_o =surface area and Alk = total alkalinity ($\text{HCO}_3^- + \text{CO}_3^{2-}$).

2.5 References

- Alberta Environmental Protection. 1998. The Boreal Forest Natural Region of Alberta: Report for Special Places 2000 Provincial Coordinating Committee. Natural Resources Service, Recreation and Protected Areas Division, Natural Heritage Planning and Evaluation Branch. P. 261.
- Armstrong, F.A.J., Sterns, C.R. and J.D.H. Strickland. 1967. The measurement of upwelling and subsequent biological processes by means of the Technicon auto-analyzer and associated equipment. *Deep Sea Research* 14: 381-389.
- Arnott, S.E., J.J. Magnuson and N.D. Yan. 1998. Crustacean zooplankton species richness: single- and multiple-year estimates. *Canadian Journal of Fisheries and Aquatic Sciences* 55: 1573-1582.
- Arnott, S.E., N.D. Yan, J.J. Magnuson and T.M. Frost. 1999. Interannual variability and species turnover of crustacean zooplankton in Shield lakes. *Canadian Journal of Fisheries and Aquatic Sciences* 56: 162-172.
- Bayly, I.A.E. 1993. The fauna of athalassic saline waters in Australia and the Altiplano of South America: comparisons and historical perspectives. *Hydrobiologia* 267: 225-231.
- Beadle, L.C. 1969. Osmotic regulation and the adaptation of freshwater animals to inland saline waters. *Verhandlungen Internationale Vereinigung für Limnologie* 17: 421-429.
- Bergmann, M. and R.H. Peters. 1980. A simple reflectance method for the measurement of particulate pigment in lake water and its application to phosphorus-chlorophyll-seston relationships. *Canadian Journal of Fisheries and Aquatic Sciences* 37: 111-114.
- Bierhuizen, J.F.H. and E.E. Prepas. 1985. Relationship between nutrients, dominant ions, and phytoplankton standing crop in prairie saline lakes. *Canadian Journal of Fisheries and Aquatic Sciences* 42: 1588-1594.
- Blinn, D.W. 1993. Diatom community structure along physicochemical gradients in saline lakes. *Ecology* 74: 1246-1263.
- Bos, D.G., B.F. Cumming and J.P. Smol. 1999. Cladocera and Anostraca from the Interior Plateau of British Columbia, Canada, as paleolimnological indicators of salinity and lake level. *Hydrobiologia* 392: 129-141.

- Bos, D.G., B.F. Cumming, C.E. Watters and J.P. Smol. 1996. The relationship between zooplankton, conductivity and lake-water ionic composition in 111 lakes from the Interior Plateau of British Columbia, Canada. *International Journal of Salt Lake Research* 5: 1-15.
- Brandlova, J. Z. Brandl and C.H. Fernando. 1972. The Cladocera of Ontario with remarks on some species and distribution. *Canadian Journal of Zoology* 50: 1373-1403.
- Brooks, J.L. 1957. The Systematics of North American *Daphnia*. *Memoirs of the Connecticut Academy of Arts and Sciences* 13: 1-180.
- Burland, G.R., undated. An identification guide to Alberta aquatic plants. Alberta Environmental Protection, Environmental Regulation Services, Pest Management Branch, Edmonton. 17 pp.
- Canadian Parks Service. 1992. World heritage: Canadian World Heritage sites. Minister of Supply and Services Canada, Ottawa. Cat. No. R62-152/1992. ISBN 0-662-58785-5.
- Campbell, C.E. and E.E. Prepas. 1986. Evaluation of factors related to the unusually low chlorophyll levels in prairie saline lakes. *Canadian Journal of Fisheries and Aquatic Sciences* 43: 846-854.
- Camsell, C. 1917. Salt and gypsum deposits in district between Peace and Slave rivers, northern Alberta. Geological survey of Canada summary report 1916. Pp. 134-145.
- Caraco, N.F., J.J. Cole and G.E. Likens. 1989. Evidence for sulphate-controlled phosphorus release from sediments of aquatic systems. *Nature* 341: 316-318.
- Chengalath, R., C.H. Fernando and M.G. George. 1971. Planktonic Rotifera of Ontario. Dept. of Biology, University of Waterloo, Ont. Biology series. 40 pp.
- Colbourne, J.K. and P.D.N. Hebert. 1996. The systematics of North American *Daphnia* (Crustacea: Anomopoda): a molecular phylogenetic approach. *Philosophical Transactions of the Royal Society of London B* 351: 349-360.
- Covich, A.P., C. Fritz, P.J. Lamb, R.D. Marzolf, W.J. Matthews, K.A. Poiani, E.E. Prepas, M.B. Richman and T.C. Winter. 1997. Potential effects of climate change on aquatic ecosystems of the Great Plains of North America. *Hydrological Processes* 11: 993-1021.
- Cuthbert, I.D. and P. del Giorgio. 1992. Toward a standard method of measuring color in freshwater. *Limnology and Oceanography* 37: 1319-1326.

- Devey, Jr. E.S., and G.B. Devey. 1971. The American species of *Eubosmina seligo* (Crustacea, Cladocera). *Limnology and Oceanography* 16: 201-218.
- de Villiers, M. 1999. *Water*. Stoddart Publishing Company Ltd., Toronto. 413 pp.
- Dodson, S.I. 1981. Morphological variation of *Daphnia pulex* Leydig (Crustacea: Cladocera) and related species from North America. *Hydrobiologia* 83: 101-114.
- Edmondson, W.T. 1959. *Freshwater Biology*. John Wiley and Sons Inc., New York.
- Evans, J.C. and E.E. Prepas. 1996. Potential effects of climate change on ion chemistry and phytoplankton communities in prairie saline lakes. *Limnology and Oceanography* 41: 1063-1076.
- Evans, J.C. and E.E. Prepas. 1997. Relative importance of iron and molybdenum in restricting phytoplankton biomass in high phosphorus saline lakes. *Limnology and Oceanography* 42: 461-472.
- Evans, M.S., R.D. Robarts and M.T. Arts. 1995. Predicted versus actual determinations of algal production, algal biomass, and zooplankton biomass in a hypereutrophic, hypersaline prairie lake. *Canadian Journal of Aquatic Sciences* 52: 1037-1049.
- Frey, D.G. 1993. The penetration of cladocerans into saline waters. *Hydrobiologia* 267: 233-248.
- Galat, D.L. and R. Robinson. 1983. Predicted effects of increasing salinity on the crustacean zooplankton community of Pyramid Lake, Nevada. *Hydrobiologia* 105: 115-131.
- Gorham, E., W.E. Dean and J.E. Sanger. 1983. The chemical composition of lakes in the north-central United States. *Limnology and Oceanography* 28: 287-301.
- Government and the University of Alberta. 1969. *Atlas of Alberta*. University of Alberta Press in association with University of Toronto Press, Edmonton.
- Greenberg, A.E., L.S. Clesceri, and A.D. Eaton. 1992. *Standard Methods for the Examination of Water and Wastewater*, 18th ed. American Public Health Association, American Water Works Association and Water Environment Federation, Washington, D.C.
- Hammer, U.T. 1986a. *Saline Ecosystems of the World*. Dr. W. Junk Publishers, Dordrecht. 616 pp.
- Hammer, U.T. 1986b. Saline lake resources of the Canadian prairies. *Canadian Water Resources Journal* 11: 43-57.

- Hammer, U.T. 1990. The effects of climate change on the salinity, water levels and biota of Canadian prairie saline lakes. *Verhandlungen Internationale Vereinigung für Limnologie* 24: 321-326.
- Hammer, U.T. 1993. Zooplankton distribution and abundance in saline lakes of Alberta and Saskatchewan, Canada. *International Journal of Salt Lake Research* 2: 111-132.
- Hammer, U.T. and L. Forró. 1992. Zooplankton distribution and abundance in saline lakes of British Columbia, Canada. *International Journal of Salt Lake Research* 1: 65-80.
- Hammer, U.T., R.C. Haynes, J.M. Heseltine and S.M. Swanson. 1975. The saline lakes of Saskatchewan. *Verhandlungen Internationale Vereinigung für Limnologie* 19: 589-598.
- Horne, A.J. and D.L. Galat. 1985. Nitrogen fixation in an oligotrophic, saline desert lake: Pyramid Lake, Nevada. *Limnology and Oceanography* 30: 1229-1239.
- Ivanova, M.B. 1990. Zooplankton of highly saline lakes. *Gidrobiologicheskii zhurnal* 26: 3-9.
- Jellison, R., L.G. Miller, J.M. Melack and G.L. Dana. 1993. Meromixis in hypersaline Mono Lake, California. 2. Nitrogen fluxes. *Limnology and Oceanography* 38: 1020-1039.
- Jongman, R.H.G., C.J.F. ter Braak and O.F.R. van Tongeren. *Data Analysis in Community and Landscape Ecology*. 1987. Centre for Agricultural Publishing and Documentation, Pudoc Wageningen. 299 pp.
- Joye, S.B., T.L. Connell, L.G. Miller, R.S. Oremland and R.S. Jellison. 1999. Oxidation of ammonia and methane in an alkaline, saline lake. *Limnology and Oceanography* 44: 178-188.
- Keller, W. and M. Conlin. 1994. Crustacean zooplankton communities and lake morphometry in Precambrian Shield lakes. *Canadian Journal of Fisheries and Aquatic Sciences* 51: 2424-2434.
- Koel, T.M. and J.J. Peterka. 1995. Survival to hatching of fishes in sulfate-saline waters, Devils Lake, North Dakota. *Canadian Journal of Fisheries and Aquatic Sciences* 52: 464-469.
- Larsen, C.P.S. 1997. Spatial and temporal variations in boreal forest fire frequency in northern Alberta. *Journal of Biogeography* 24: 663-673.

- Last, W.M. 1992. Chemical composition of saline and subsaline lakes of the northern Great Plains, western Canada. *International Journal of Salt Lake Research* 1: 47-76.
- Last, W.M. and T.H. Schweyen. 1983. Sedimentology and geochemistry of saline lakes of the Great Plains. *Hydrobiologia* 105: 245-263.
- Legendre, P. and L. Legendre. 1998. *Numerical Ecology*, 2nd ed. Developments in Environmental Modelling 20, Elsevier Science B.V., Amsterdam. Pp. 575-635.
- Leland, H.V. and W.R. Berkas. 1998. Temporal variation in plankton assemblages and physicochemistry of Devils Lake, North Dakota. *Hydrobiologia* 377: 57-71.
- Lemmen, D.S., A. Duk-Rodkin and M.J. Bednarski. 1994. Late glacial drainage systems along the northwestern margin of the Laurentide ice sheet. *Quaternary Science Reviews* 13: 805-828.
- Meijer Drees, N.C. 1986. Evaporitic deposits of Western Canada. Geological survey of Canada. Paper 85-20. Pp 45, 51.
- Menzel, D.W. and N. Corwin. 1965. The measurement of total phosphorus in seawater based on the liberation of organically bound fractions by persulfate oxidation. *Limnology and Oceanography* 10: 280-282.
- Moser, K.A., J.P. Smol, D.R.S. Lean and G.M. MacDonald. 1998. Physical and chemical limnology of northern boreal lakes, Wood Buffalo National Park, northern Alberta, and the Northwest Territories, Canada. *Hydrobiologia* 377: 25-43.
- Nelson, J.S. and M.J. Paetz. 1992. *The Fishes of Alberta*. University of Alberta Press, Edmonton. 437 pp.
- Patalas, K. and A. Salki. 1993. Spatial variation of crustacean plankton in lakes of different size. *Canadian Journal of Fisheries and Aquatic Sciences* 50: 2626-2640.
- Pienitz, R., J.P. Smol and D.R.S. Lean. 1997a. Physical and chemical limnology of 24 lakes located between Yellowknife and Contwoyto Lake, Northwest Territories (Canada). *Canadian Journal of Fisheries and Aquatic Sciences* 54: 347-358.
- Pienitz, R., J.P. Smol and D.R.S. Lean. 1997b. Physical and chemical limnology of 59 lakes located between the southern Yukon and the Tuktoyaktuk Peninsula, Northwest Territories (Canada). *Canadian Journal of Fisheries and Aquatic Sciences* 54: 330-346.

- Pienitz, R., I.R. Walker, B.A. Zeeb, J.P. Smol and P.R. Leavitt. 1992. Biomonitoring past salinity changes in an athalassic subarctic lake. *International Journal of Salt Lake Research* 1: 91-123.
- Pinel-Alloul, B., T. Niyonsenga and P. Legendre. 1995. Spatial and environmental components of freshwater zooplankton structure. *Ecoscience* 2: 1-19.
- Prepas, E.E. 1978. Sugar-frosted *Daphnia*: an improved fixation technique for Cladocera. *Limnology and Oceanography* 23: 557-559.
- Prepas, E.E. and F.H. Rigler. 1982. Improvements in quantifying the phosphorus concentration in lake water. *Canadian Journal of Fisheries and Aquatic Sciences* 39: 822-829.
- Prepas, E.E. and D.O. Trew. 1983. Evaluation of the phosphorus-chlorophyll relationship for lakes off the Precambrian Shield in western Canada. *Canadian Journal of Fisheries and Aquatic Sciences* 40: 27-35.
- Pfaff, J.D. 1993. Determination of inorganic anions by ion chromatography, revision 2.1, EPA method 300.0. United States Environmental Protection Agency Environmental Monitoring Systems Laboratory, Office of Research and Development, Cincinnati, OH 45268.
- Rawson, D.S. and A.J. Moore. 1944. The saline lakes of Saskatchewan. *Canadian Journal of Research D*. 22: 141-201.
- Renaut, R.W. 1990. Recent carbonate sedimentation and brine evolution in the saline lake basins of the Cariboo Plateau, British Columbia, Canada. *Hydrobiologia* 197: 67-81.
- Reynolds, J.D. 1979. Crustacean zooplankton of some saline lakes of central British Columbia. *Syesis* 12: 169-173.
- Rühland, K. and J.P. Smol. 1998. Limnological characteristics of 70 lakes spanning arctic treeline from Coronation Gulf to Great Slave Lake in the Central Northwest Territories, Canada. *International Reviews in Hydrobiologie* 83: 183-203.
- Scudder, G.G.E. 1983. A review of factors governing the distribution of two closely related corixids in the saline lakes of British Columbia. *Hydrobiologia* 105: 143-154.
- Smith, D.G. 1994. Glacial Lake McConnell: paleogeography, age, duration, and associated river deltas, MacKenzie River basin, Western Canada. *Quaternary Sciences Reviews* 13: 829-843.

- Smith, K. and C.H. Fernando. 1978. A Guide to the Freshwater Calanoid and Cyclopoid Copepod Crustacea of Ontario. Dept. of Biology, University of Waterloo, Ont. Biology series. 74 pp.
- SPSS Inc. 1997. Sigmastat 2.0 for Windows. Marketing Department, 444 North Michigan Ave., Chicago IL 60611.
- Stainton, M.P., M.J. Capel and F.A.J. Armstrong. 1977. The chemical analysis of freshwater, 2nd ed. Fish. Environ. Can. Misc. Spec. Publ. 25: 180. Available on request from the Freshwater Institute, Winipeg, MN.
- Stemberger, R.S. 1979. A Guide to Rotifers of the Laurentian Great Lakes. U.S. Environmental Protection Agency Rep. No. EPA-600-14-79-021. 185 pp.
- Stemberger, R.S. and J.M. Lazorchak. 1994. Zooplankton assemblage responses to disturbance gradients. Canadian Journal of Fisheries and Aquatic Sciences 51: 2435-2447.
- ter Braak, C.J.F. and P. Šmilauer. 1998. CANOCO 4.0 Reference Manual and User's Guide to Canoco for Windows: software for canonical community ordination (version 4.0). Microcomputer Power, Ithaca, NY. 351 pp.
- Tsui, P.C. 1982. Deformation, ground subsidence, and slope movements along the Salt River Escarpment in Wood Buffalo National Park. M.Sc. thesis, Department of Geology, University of Alberta. 140 pp.
- Toth, J. 1999. Groundwater as a geologic event: an overview of the causes, processes, and manifestations. Hydrogeology Journal 7: 1-14.
- Topping, M.S. and G.G.E. Scudder. 1977. Some physical and chemical features of saline lakes in central British Columbia. Syesis 10: 145-166.
- Vallentyne, J.R. 1972. Freshwater supplies and pollution: effects of the demophoric explosion on water and man. In: N. Polunin (ed.). The Environmental Future. MacMillan Press, London. Pp. 181-211.
- Waiser, M.J. and R.D. Robarts. 1995. Microbial nutrient limitation in prairie saline lakes with high sulfate concentration. Limnology and Oceanography 40: 566-574.
- Walker, K.F. 1975. The seasonal phytoplankton cycles of two saline lakes in central Washington. Limnology and Oceanography 20: 40-53.
- Wetzel, R.G. 1983. Limnology, 3rd ed. Saunders College Publishing, Toronto.

- Williams, W.D., A.J. Boulton and R.G. Taaffe. 1990. Salinity as a determinant of salt lake fauna: a question of scale. *Hydrobiologia* 197: 257-266.
- Williams, W.D. 1993. The worldwide occurrence and limnological significance of falling water-levels in large, permanent saline lakes. *Verhandlungen Internationale Vereinigung für Limnologie* 25: 980-983.
- Williams, W.D. 1998. Salinity as a determinant of the structure of biological communities in salt lakes. *Hydrobiologia* 381: 191-201.
- Wilson, S.E., B.F. Cumming and J.P. Smol. 1994. Diatom-salinity relationships in 111 lakes from the Interior Plateau of British Columbia, Canada: the development of diatom-based models for paleosalinity reconstructions. *Journal of Paleolimnology* 12: 197-221.
- Winter, T.C. 1977. Classification of the hydrologic settings of lakes in the North Central United States. *Water Resources Research* 13: 753-767.
- Winter, T.C. 1989. Distribution of the difference between precipitation and open-water evaporation in North America, *Surface Water Hydrology*. In: U.S. Geological Survey (ed.). *The Geology of North America V. 0-1*. The Geological Society of America, Inc., Boulder, Colorado. Plate 2.
- Wintermans, J.F.G.M. and A. DeMots. 1965. Spectrophotometric characteristics of chlorophyll a and b and their phaeophytins in ethanol. *Biochimica et Biophysica Acta* 109: 448-453.
- Wood Buffalo National Park. 1999. Data from Benchmark Creek weather station, Wood Buffalo National Park, Fort Smith, Northwest Territories.
- Wurtsbaugh, W.A. and T.S. Berry. 1990. Cascading effects of decreased salinity on the plankton, chemistry, and physics of the Great Salt Lake (Utah). *Canadian Journal of Fisheries and Aquatic Sciences* 47: 100-109.
- Zotina, T.A., A.P. Tolomeyev and N.N. Degermendzhy. 1999. Lake Shira, a Siberian salt lake: ecosystem structure and function. I. Major physico-chemical and biological features. *International Journal of Salt Lake Research* 8: 211-232.

**CHAPTER 3:
EVOLUTION OF MONOGONONT ROTIFERS:
A MOLECULAR PHYLOGENETIC APPROACH**

3.0 Introduction

Evolutionary relationships within the Phylum Rotifera are poorly understood despite the important role that many taxa play in aquatic ecosystem dynamics (Wallace and Snell 1991). Members of the class Monogononta (Appendix A) often dominate freshwater zooplankton communities (MacIsaac et al. 1987) and represent 90% of the species recognized within the phylum (Pennak 1989). The morphological differentiation of these species has proven complicated because of the extensive phenotypic variation caused by environmental and genetic factors (Serra et al. 1998). In addition, the alternation of asexual and bisexual reproduction coupled with extremely short generation times is thought to promote the genetic divergence of rotifer populations that may occur in the absence of morphological change (Serra et al. 1997). The processes that cause genetic differentiation of populations are subject to considerable debate. Divergence among populations can be caused by founder effects and genetic drift (stochastic events), and from natural selection acting on adaptive variation (Hartl and Clark 1997). Application of molecular techniques would assist in clarifying species boundaries among rotifers (Walsh 1993).

3.0.1 *Phenotypic Variation Among Species*

Cyclomorphosis refers to seasonal phenotypic alterations in a single population that are related to biological, chemical, or physical environmental cues (Wallace and Snell 1991). Examples include modified posterior spine length in species of *Keratella* in response to variation in predation regimes (e.g. Stemberger and Gilbert 1984), competition (Stemberger and Gilbert 1987), as well as food concentration and

temperature (e.g. Hillbricht-Ilkowska 1983). Phenotypic plasticity is further complicated by annual cyclomorphosis, which has been observed in *Keratella cochlearis* and *Keratella quadrata*, in which morphs with long posterior spines initiate the cycle and there is progressive reduction in spine length in successive asexual generations (Pennak 1989). The establishment of clear species boundaries is difficult because phenotypic variability induced by environmental mechanisms can be confused with genetic changes in populations, such as clonal replacements or seasonal successions of species (Kerfoot 1980). Complexes of sibling species have been suggested for *K. cochlearis* (Hofman 1983) and identified for *Brachionus plicatilis* (Gomez and Snell 1996).

3.0.2 Genetic Divergence Within Species

In spite of their potential for widespread genetic exchange through passive dispersal of resting stages (e.g. Weider et al. 1996, Weider et al. 1999), genetic differentiation among zooplankton populations often occurs on a local scale (De Meester 1996, Colbourne et al. 1997). Most zooplankton hatch in the same lake in which their resting eggs were produced (De Meester 1996), and the spatial boundaries set by lakes and ponds are thought to restrict gene flow between populations (Slatkin 1985). Monogononts may have high population divergences because of their minute size (most 100-500 μm), rapid multiplication rates resulting from cyclic parthenogenesis, and phenomenal numbers (Serra et al. 1997). Generation times as short as 1 generation every two days (Miracle and Serra 1989) may promote genetic divergence within species because DNA is copied often, resulting in an accelerated accumulation of mutations (e.g. Hafner et al. 1994) during both the asexual and sexual phase of reproduction (Gomez and Carvalho 2000). The colonization of temporally variable environments may

influence the timing of sexual reproduction and enhance genetic differentiation across geographic areas (Serra et al. 1997). There is evidence that salinity may contribute to spatial and temporal gradients in aquatic environments that can promote intraspecific divergence of cyclic parthenogens (Weider and Hebert 1987, Wilson and Hebert 1992). Additionally, accelerated rates of molecular evolution have been reported for saline lake fauna, such as brine shrimp (Iwabe et al. 1996, Maley and Marshall 1998) and halophilic cladocerans (Hebert 1998, Hebert et al. 2001).

3.0.3 Application of Molecular Systematics

Few molecular studies have examined evolutionary relationships among or within monogonont rotifer species, and most existing knowledge derives from allozyme studies. Ecological factors that have been found to interact with the genetic structure of rotifer populations include photoperiod, population density, food quality, salinity (King 1980), cyanobacterial blooms (Snell 1980), and chemical toxicants (Snell et al. 1991a). Other allozyme work has shown that *B. plicatilis* is a complex of at least three co-occurring sibling species in a coastal marsh in Spain (Gomez et al. 1995), with varying ecological preferences (Gomez et al. 1997) and mating patterns (Gomez and Serra 1995) associated with different salinities and temperatures. There was evidence for more sibling species in the *B. plicatilis* complex when strains from different parts of the world were analyzed (Gomez and Snell 1996). PCR-based microsatellites have been employed to show that genetic diversity within cyclic parthenogenetic rotifer populations is maintained by sexually-produced resting eggs (Gomez and Carvalho 2000). Gomez et al. (2000b) recently produced the first mitochondrial DNA (mtDNA) phylogeny of rotifers for *B. plicatilis* strains collected from salt lakes on the Iberian Peninsula, Spain and showed

considerable genetic divergence (2.8% uncorrected COI nucleotide sequence divergence) between southern and northern populations.

The objectives of this study were to 1) address whether there were genetic clades within and among selected *Keratella* species that had been previously masked by phenotypic plasticity 2) determine if there is intraspecific genetic divergence of salt-tolerant rotifers (*Brachionus plicatilis*) across lakes spanning a salinity gradient in northern Canada. Mitochondrial genes 16S rRNA and cytochrome oxidase I (COI) were employed to build molecular phylogenies to determine the degree of nucleotide sequence divergence within these two genera, which are known for having highly variable morphologies or species complexes. I hypothesized that genetic clades previously masked by phenotypic plasticity would be detected in *Keratella* sp. and that greater genetic variability would be detected between populations of saltwater than freshwater species. Additionally, I predicted that substantial intraspecific genetic structure would be detected for *B. plicatilis* because of the possibility of cryptic species at different lakewater salinities. MtDNA sequences of *B. plicatilis* strains from Spain (Gomez et al. 2000b) were included in the phylogenetic analysis of Canadian strains to provide a more comprehensive understanding of population divergence within this species. A species of *Synchaeta* was included in the phylogenies to estimate divergence between the families Brachionidae (*Keratella* and *Brachionus*) and Synchaetidae, as well as to assess the feasibility of future mtDNA work on this genus. This study provides baseline information for future investigations in the molecular phylogenetics of rotifers.

3.1 Materials and Methods

3.1.1 Field Collection and Identifications

Rotifers were collected at the point of maximum lake depth with a conical 64 μm Nitex tow net from nine lakes that spanned a salinity gradient (556 to 26 318 mg/l total dissolved solids (TDS), summer means). These lakes were located within 85 km of each other in Wood Buffalo National Park (WBNP), Alberta (59°30'N, 111°80'W) (Figure 3-1). In addition, *Brachionus urceolaris*, *Keratella cochlearis robusta*, and one *Keratella quadrata* population were obtained from mine-tailings reclamation ponds 330 km south of the study lakes, near Fort McMurray, Alberta (56°55'N, 111°29'W) (Figure 3-1). Individuals of *B. calyciflorus* from Gainesville, Florida (29°N, 82°W) (Snell et al. 1991b) were used as a freshwater reference for other *Brachionus* species. Members of the genus *Asplanchna* sp. were collected from southern Ontario (44°N, 80°W) and served as an outgroup in the phylogenetic analyses. Vertical net hauls were performed in lakes that were sufficiently deep (2 to 10 m) for vertical migration of zooplankton to occur while horizontal tows were conducted in shallow (0.3 to 0.5 m) lakes. Live rotifers were transported from the field to the lab where they were identified to genus for *Synchaeta* sp. and to species for other the taxa using keys in Stemberger (1979). Total DNA was extracted from individuals using proteinase K methods (Schwenk 1996), and extractions were stored frozen at -20°C until further genetic analysis.

3.1.2 Molecular Protocols

Two mitochondrial (mt) genes with different rates of molecular evolution were employed to screen for genetic variability within and between selected rotifer families. Cytochrome oxidase subunit I (COI) was used to investigate variation among conspecific

populations while 16S rDNA (16S) was used to examine deeper phylogenetic relationships (Palumbi 1996). The polymerase chain reaction (PCR) (Saiki et al. 1998) was used to amplify a 447 base pair (bp) fragment of the 16S gene with the primer pair 16S-AR and 16S-BR (Palumbi 1996). Each 50 µl PCR reaction contained 7 µl of DNA template, 4.5 µl of 10x PCR buffer (Roche), 2.2 µl of 50 µM MgCl₂, 0.25 µl of each 10 µM dNTP (C, G, A, T), 1 µl of each 10 µM primer, 0.4 µl of 1:10 *Taq* DNA polymerase (Qiagen Inc), and 32.9 µl of sterile, double-distilled water. PCR was also employed to amplify a 633 bp fragment of cytochrome c oxidase subunit I with LCOI490 and HCO2918 primers described by Folmer et al. (1994). Each 50 µl PCR reaction used to amplify the COI fragment consisted of the same ingredients as 16S, with the exception of 3 µl of DNA template and 36.9 µl of sterile, double-distilled water. Procedures for PCR amplification involved 1 cycle of 1 minute at 94°C; 40 cycles of 1 min. at 94°C, 1.5 min. at 45°C, and 1.5 min at 72°C; followed by 1 cycle of 5 min. at 72°C in an MJ Research PCR machine (PTC-100). PCR products were gel-purified (1.6% agarose) using the Qiaex II kit (Qiagen Inc). DNA fragments were sequenced with an ABI Prism 377 automated sequencer (Applied Biosystems), with primers 16S-AR and LCO1490 respectively, and the *Taq* FS dye rhodamine sequencing kit (Perkin-Elmer). DNA sequences were checked for accidental amplification of contaminating agents by searching the Genbank/EMBL database (Altschul et al. 1997).

3.1.3 Sequence and Phylogenetic Analysis

Non-protein coding 16S sequences were aligned with Sequence Navigator (ABI Prism, Applied Biosystems Inc., Perkin Elmer) using the Clustal alignment default parameters. Segments of the 16S gene were omitted where alignments were ambiguous

as a result of gap hypervariability. Fragments of 387 bp were consequently used in phylogenetic analyses of the 16S sequence data. For protein-coding COI, sequence electropherograms were aligned with the Seqapp 1.9a sequence editor using default parameters (Gilbert 1992), and an unambiguous alignment was produced because of the absence of gaps. Average nucleotide diversity was calculated according to Hartl (2000) for selected interspecific comparisons. COI nucleotide sequences of 627 bp translated to 209 amino acids (*Drosophila* mtDNA code) in MEGA 1.02 (Kumar et al. 1993). COI sequences belonging to *Brachionus plicatilis* haplotypes from the Iberian Peninsula, Spain were obtained from GenBank and were included in phylogenetic analyses.

Phylogenetic trees were generally rooted with an outgroup taxon (*Asplanchna* sp.) of a species belonging to the same order but a different family than the family under study. For comparison of intraspecific divergence between *B. plicatilis* haplotypes, phylogenetic trees were rooted with a closely related freshwater species, *B. calyciflorus*. This was done in order to reduce the amount of homoplasy that occurs along long branches when distantly related taxa are used as outgroups, which can result in problems associated with “long-branch attraction” (Swofford et al. 1996). Homoplasy refers to similarity between nucleotide sequences that is a result of their independent acquisition rather than shared ancestry. Gaps in the 16S sequences were treated as missing data because of the difficulty of modelling insertions and deletions associated with the secondary structure of 16S rRNA (Wuyts et al. 2001).

Multiple substitutions at a given nucleotide site can cause homoplasy, which can obscure attempts to recover evolutionary relationships among sequences (Page and Holmes 1998). To determine the potential impact of homoplasy on the estimation of

phylogenies from pairwise sequence comparisons, transition/transversion (Ts/Tv) ratios for 16S and COI were plotted against percent sequence divergence. Transitions occur more frequently than transversions in mtDNA, particularly at the third codon position (Xia et al. 1996). As sequence divergence increases, transitions can become saturated with respect to transversions (Ts/Tv=1), and cause homoplasy. For 16S, a plot of Ts/Tv ratios versus percent sequence divergence indicated that neither transitions nor transversions were saturated (Appendix B), and all characters were treated as unordered and equally weighted. Transitions were also not saturated for COI *Brachionus plicatilis* haplotypes (Appendix C) and consequently, characters were equally weighted. In contrast, transitions appeared to be saturated for COI when all taxa were included in the analysis (Appendix D), and transversions were given twice as much weight as transitions to reduce homoplasy. However, this weighting resulted in the mixing of families and genera, and also produced a tree of greater length (score=1369) than the unweighted data (score=853). Consequently, I treated COI characters as unordered and equally weighted despite the apparent saturation of transitions when all taxa were included in the phylogenetic analysis.

Analyses were conducted with PAUP 4.02b (Swofford 1998) except where noted otherwise. A chi-square goodness-of-fit test was performed on the sequence data for each gene region to determine if shifts in nucleotide composition occurred among taxa. Whereas base frequencies were homogeneous among 16S sequences (homogeneity, $\chi^2=15.22$, $df=45$, $p>0.99$), COI nucleotide base composition was variable (homogeneity, $\chi^2=397.32$, $df=129$, $p<0.01$) when all taxa from this study were included. COI base composition was homogeneous for intraspecific comparisons within *B. plicatilis*

including data from GenBank (homogeneity, $X^2=37.40$, $df=111$, $P>0.99$). The Kimura 2-parameter distance model (Kimura 1980) was employed to correct for the possibility of multiple superimposed substitution events in the 16S and *B. plicatilis* COI sequences because the assumption of homogeneous base composition was met and there was no evidence of transitional saturation (Kumar et al. 1993). This simple measure was chosen because more complex distance models yielded similar topologies for phylogenetic trees, and variances are lower when fewer parameters are estimated. The model of Tamura and Nei (1993) was employed to correct pairwise differences between COI sequences involving all of the selected taxa from this study. This model compensates for nucleotide base heterogeneity and transition/transversion bias (Kumar et al. 1993), and is a compromise between computability and accuracy (Reed and Sperling 1999). Matrices of distance measures with standard errors were determined using MEGA 1.02 (Kumar et al. 1993).

Both phenetic (distance-based) and cladistic (character-based) analyses were employed in the phylogenetic studies. Phenetic analyses calculate overall change or divergence between pairwise sequences, but do not indicate where in the tree each site changes. Phenetics are based on a measure of similarity between organisms and do not imply a direction for evolutionary change. Neighbour-joining (NJ) employs clustering to phenetically estimate the tree with the smallest sum of branch lengths (minimum evolution tree) using pairwise distances between the sequences, and does not assume a molecular clock (Page and Holmes 1998). Matrices of distance measures were used to estimate NJ phenograms with confidence limits determined with 1000 bootstrap pseudoreplicates for 16S rDNA and COI nucleotide sequences. Bootstrap analysis was

only performed for COI amino acid trees at levels above the species because of reduced phylogenetic signal in intraspecific comparisons, which is a consequence of the conserved amino acid gene product. Bootstrap values obtained from bootstrap concencuses of distance trees were applied to nodes in the cladistic trees.

Cladistic analyses directly examine the sequence differences rather than pairwise distances, and use the similarity of individual nucleotide sites between two or more taxa to infer patterns of ancestry. Maximum parsimony (MP) is a cladistic technique that searches for the tree or trees that have the fewest evolutionary changes (minimum evolution tree) based on the fewest substitutions that could have occurred at each individual nucleotide site (Page and Holmes 1998). Cladistic analyses were not performed for amino acid diversity in *B. plicatilis* because parsimony-informative characters were absent. MP analysis of phylogenetically informative sites employed heuristic searches with a starting tree obtained by 1000 replicates of random stepwise sequence addition. Optimal trees were found with the tree bisection-reconnection (TBR) branch swapping algorithm and the MulTrees and steepest descent options invoked in PAUP 4.02b. Groups appearing in $\geq 70\%$ of the replicates were considered well-supported (Hillis and Bull 1993). Confidence in the cladistic analyses were assessed, both *a priori*, by estimation of the g_1 skewness statistic from 100 000 random tree length distributions (Hillis and Heulsenbeck 1992), and *a posteriori* by bootstrap analysis with 1000 pseudoreplicates. Homoplasly was measured in MP analyses with the homoplasly index (HI) which, similar to the consistency index (CI), may be subject to increased homoplasly levels in association with increased taxa and character number (Archie 1989).

3.2 Results

3.2.1 16S: Inter-familial and Inter-specific Comparisons of Selected Taxa

3.2.1.1 Sequence Variability

The sequence alignment length after hypervariable gap regions were removed was 387 base pairs (bp), of which 150 bp were variable and 113 bp were phylogenetically informative using cladistic criteria. Mean base frequencies among the rotifer genera were 0.32 (A), 0.14 (C), 0.18 (G), and 0.36 (T), and there was no evidence of heterogeneity in nucleotide composition (homogeneity $\chi^2=15.22$, $df=45$, $p>0.99$). The number of 16S haplotypes per species, the number of individuals sequenced per haplotype, and locations of sample collection are listed in Table 3-1.

3.2.1.2 Phylogenetic Analyses

In the neighbour-joining (NJ) phenogram of the 16S sequence data, resolution of the two families and three genera were congruent with prior morphological assignments (Appendix E). Members of the families Brachionidae (clades A and B) and Synchaetidae (clade C) showed an average of 23.0 % sequence divergence. Within the Brachionidae, *Brachionus* sp. (clade A) and *Keratella* sp. (clade B) showed a mean divergence of 22.1 %. Divergence between families was similar to the divergence values between the two genera within the Brachionidae because of the close association of *Brachionus* sp. with *Synchaeta* sp. compared to *Keratella* sp. (Table 3-2). Freshwater (*B. calyciflorus* and *B. urceolaris*) and saltwater (*B. plicatilis*) species of *Brachionus* differed by 8.0%, while the two freshwater species were 4.1% divergent. *Keratella cochlearis* with a single posterior spine were divergent from members of the genus with one or two posterolateral spines (*K. hiemalis* and *K. quadrata*) with a mean sequence divergence of 17.3%.

K. hiemalis was 10.7% divergent from *K. quadrata*. Mean percent nucleotide divergences \pm standard errors between phylogenetic clusters of rotifers detected by 16S according to NJ are presented in Table 3-2.

The g_i skewness statistic was highly significant ($g_i = -0.80$, $g_{i\text{crit}} = -0.20$, $p = 0.01$), indicating strong phylogenetic signal in the 16S data set. Maximum parsimony heuristic searches were conducted with one thousand random addition search replicates, which yielded 2 equally parsimonious trees of length 297 (Consistency Index (CI)=0.72, Homoplasy Index (HI)=0.28, Retention Index (RI)=0.83). The 2 maximum parsimony (MP) trees were equivalent with respect to the positions of clades, and differed only in branch arrangement of haplotypes-2 and -5 of *B. plicatilis*, and this variation was collapsed in the 70% majority rule tree (Figure 3-2). The clusters identified in the NJ analysis (Appendix E) formed monophyletic clades with similar tree topologies in the MP analysis (Figure 3-2). Both the phenetic and cladistic analyses were congruent with respect to deep internal nodes, and the support for all nodes was strong.

3.2.2 COI: Inter and Intra-specific Comparisons of Selected Taxa

3.2.2.1 Sequence Variability

The number of COI haplotypes identified per species, replicate sequences per haplotype and locations of sample collection are reported in Table 3-3. Sequence alignments and amino acid translations were unambiguous, as there were no gaps or nonsense codons among the 84 COI sequences in this study. The aligned sequence corresponded to nucleotide position 1543 to 2173 of *Drosophila yakuba* (Folmer et al. 1994). An amino acid insertion was present at position 2009 of the *Drosophila yakuba* nucleotide sequence, in all rotifers examined in this study, a result previously reported by

Gomez et al. (2000b). The length of the nucleotide sequence alignment was 630 bp, of which 294 were variable for distance-based analysis and 284 were phylogenetically informative using cladistic criteria. Mean base frequencies among the rotifer genera were 0.21 (A), 0.20 (C), 0.18 (G), and 0.40 (T) but there was heterogeneity in nucleotide composition among the rotifer species (homogeneity, $X^2=397.32$, $df=129$, $P<0.001$). Nucleotide sequences translated to 210 amino acids according to the *Drosophila* mtDNA code, of which 54 nucleotide positions were variable and 51 were phylogenetically informative according to MP.

3.2.2.2 *Phylogenetic Analyses*

In the NJ phenogram for the COI nucleotide sequence data, resolution of two families (clades A, B versus clade C) and three genera (*Brachionus*: clade A, *Keratella*: clade B and *Synchaeta*: clade C) were congruent with morphological evidence (Appendix F). Average nucleotide sequence divergence between families Brachionidae (clades A and B) and Synchaetidae (clade C) was 28.9%, and the two genera representing Brachionidae differed by 28.6%. Within-family divergence of Brachionidae was similar to between-family divergence with Synchaetidae because freshwater *Brachionus* species (*B. calyciflorus* and *B. urceolaris*) appeared to have a close association with Synchaetidae (24.8%). Freshwater and saltwater *Brachionus* species had a sequence divergence of 20.0%. Unexpectedly, *K. hiemalis* appeared to be more closely related to *K. cochlearis* (25.6%) than *K. quadrata* (23.7%), which was not observed in the 16S NJ tree. Pairwise nucleotide sequence divergence between selected species of *Brachionus* ranged from 20 to 25%, *Keratella* ranged from 24 to 27%, and *Synchaeta* isolates ranged from 6 to 21% (Table 3-4).

The divergences between haplotypes of *Brachionus plicatilis* ranged from 0.3 to 1.3%, with haplotypes-15 and -16 demonstrating the greatest mean divergence (0.9%), closely followed by haplotypes *B. plicatilis* -4 to -14 (0.8%). *B. plicatilis* haplotypes clustered according to the lakes from which they were collected. Clades a and b corresponded to HC Lake, clade c to FP Lake and clade d to GL/SPL lakes (Table 3-3, Figure 3-3). Average nucleotide diversity (π) of the 16 haplotypes of *B. plicatilis* identified from 22 sequences collected from WBNP, Canada was 0.74. The mean number of non-synonymous changes resulting in amino acid differences between these haplotypes was 0.8. Clades of *Keratella cochlearis* subspecies clustered according to the presence (*K. cochlearis faluta* (f-1) and *K. cochlearis robusta* (r-1 and r-2)) or absence (*K. cochlearis tecta* (t-1 and t-2)) of posterior spines, with 4.4% mean nucleotide sequence divergence (Appendix F). Different morphs of *K. hiemalis* (one- and two-spined) showed only 0.21% divergence. *K. quadrata* showed low intraspecific nucleotide divergence, ranging from 0.2 to 0.6%. Despite the fact *K. quadrata*-5 (mean of 0.5%) was from the most geographically distant location (330 km away), it did not have greater divergence than the other haplotypes. Average nucleotide diversity (π) of 7 haplotypes identified from 27 *K. quadrata* sequences was 0.09. The mean number of non-synonymous changes between *K. quadrata* haplotypes was 0.3.

While the COI amino acid NJ phenogram (Appendix G) was congruent with 16S (Appendix E) and COI (Appendix F) nucleotide NJ trees for more recent evolutionary events, this was not the case for deeper branches on the tree. The families were not resolved according to prior morphological work. Instead, *Brachionus* sp. was monophyletic with *Synchaeta* sp. However, the three genera were resolved in the protein

NJ phenogram, and *K. hiemalis* was more closely allied with *K. quadrata* (2.3%) than *K. cochlearis* (6.5%) (Appendix G). Mean amino acid sequence divergence between families Brachionidae (clades A and B) and Synchaetidae (clade C) was 12.9%, while the two brachionid genera (*Brachionus* (clade B) and *Keratella* (clade C)) were divergent by an average of 11% (Table 3-4). Hence, the magnitude of between-family divergence was similar to the magnitude of within-family divergence. The ranges of amino acid sequence divergence between selected species of *Brachionus* sp., *Keratella* sp. and *Synchaeta* sp. isolates were 5.1 to 5.6%, 2.3 to 6.5%, and 2.8 to 5.6% respectively (Table 3-4). Unlike the 16S (Appendix E) and COI (Appendix F) nucleotide trees, clades of freshwater and saltwater *Brachionus* species were not discriminated in the amino acid tree (Appendix G). However, four protein sequence haplotypes of *B. plicatilis* were identified (clades a, b, c, d) (Appendix G), and these differed by 0.5 to 0.9%. These clades corresponded to lakes from which *B. plicatilis* haplotypes were collected (Table 3-3, Appendix G). Clusters of spined and unspined subspecies of *K. cochlearis* were 0.9% divergent. There was no amino acid divergence between different morphotypes of *K. hiemalis*, which was 2.3% divergent from *K. quadrata*, and divergence between *K. quadrata* haplotypes ranged from 0 to 0.5%.

For COI nucleotide sequence data, the g_I skewness statistic was highly significant ($g_I=-0.41$, $g_{Icrit}<-0.09$, $p=0.01$), indicating strong phylogenetic signal in the data set. One thousand random addition search replicates yielded 50 parsimonious trees of length 853 (CI=0.53, HI=0.47, RI=0.89). The 50 MP trees were equivalent with respect to positions of clades and differed only in the branch arrangement of *Synchaeta* sp. isolates and intraspecific haplotypes within *B. plicatilis* and *K. quadrata*, and this variation was

collapsed in the 70% majority rule concensus tree (Figure 3-3). The clades identified in the NJ analysis (Appendix F) formed monophyletic clades in the MP analysis and had strong bootstrap support at shallow nodes (Figure 3-3). MP and NJ analyses converged on similar tree topologies, with the exception of the placement of the genus *Synchaeta* which appeared within the family Brachionidae in the MP tree, but as a separate family in the NJ analysis. Also, the positions of *B. calyciflorus* and *B. urceolaris* were unresolved with respect to congeneric taxa in the MP tree. Furthermore, unlike the NJ phenogram, *K. hiemalis* was monophyletic with *K. quadrata* rather than *K. cochlearis*. Although both the phenetic (NJ) (Appendix F) and cladistic (MP) (Figure 3-3) analyses were congruent for all other deep internal nodes of the nucleotide sequence data, the support for these nodes was weak.

For COI amino acid sequence data, one thousand random addition search replicates yielded 1 MP tree of length 92 (CI=0.77, HI=0.23, RI=0.96). Similar to the nucleotide MP tree (Figure 3-3), the amino acid MP cladogram (Appendix H) did not resolve families Brachionidae and Synchaetidae because *Synchaeta* sp. clustered with the genera *Brachionus*. The phenetic and cladistic amino acid analyses were congruent with respect to all other deep internal nodes (Appendix G and H), and bootstrap values were higher for amino acid sequence data (Appendix H) than nucleotide sequence data (Figure 3-3).

3.2.3 COI: Intraspecific Comparisons within *Brachionus plicatilis*

3.2.3.1 Sequence Variability

Gomez et al's (2000b) dataset is comprised of 21 haplotypes of *Brachionus plicatilis* from saline lakes on the Iberian Peninsula, Spain and I have identified 16 haplotypes of this species from northern Alberta (WBNP), Canada (Table 3-3). I aligned nucleotide position 1543 to 2164 of the published *Drosophila yakuba* sequence (Folmer et al. 1994). The length of the nucleotide sequence alignment was 624 bp, of which 161 bp were variable and 54 bp were phylogenetically informative using cladistic criteria. Mean base frequencies among *B. plicatilis* haplotypes were 0.20 (A), 0.22 (C), 0.21 (T), 0.37 (G), and base composition was homogeneous (homogeneity, $\chi^2=37.40$, $df=111$, $P>0.99$). Nucleotide sequences translated to 208 amino acids according to *Drosophila* mtDNA code, of which 12 amino acid positions were variable but none were phylogenetically informative for cladistic analysis.

3.2.3.2 Phylogenetic Analyses

The NJ phenogram for COI nucleotide sequence data showed that the Spanish and Canadian populations of *B. plicatilis* were separated into two groups showing an average divergence of 4.3% (Appendix I). Two major clades were identified by Gomez et al. (2000b) within the Spanish haplotypes, which show a mean sequence divergence of 2.6%. Within each of these clades, mean sequence divergence was 0.4% and 1.3% respectively. The most distant WBNP haplotypes from Gomez's (2000b) nucleotide sequences were *B. plicatilis*-4 to -14 (clade d) (Appendix I), which had an average divergence of 4.4%. Within the Canadian haplotypes, there were three clades, of which clade d (*B. plicatilis*-4 to -14) was the most divergent from the other WBNP haplotypes,

with a mean of 0.8 %. A similar branch arrangement was obtained for WBNP *B. plicatilis* haplotypes when the Kimura 2-parameter NJ tree was rooted with *B. calyciflorus* (Appendix I) as when *Asplanchna* sp. was used as an outgroup in the Tamura-Nei NJ tree (Appendix F). The phylogenetic signal was strong for the nucleotide data ($g_1=-0.36$, $g_{1crit}<-0.12$, $p=0.01$). One thousand random addition search replicates yielded 856 parsimonious trees of length 206 (CI=0.85, HI=0.15, RI=0.92). The MP trees varied in terms of haplotype branch arrangement within the Spanish and Canadian clades, but all trees consistently resolved these two populations. The variation within these clades was collapsed in a 70% majority rule tree (Figure 3-4). Both NJ and MP analyses were congruent for the separation of Spanish and Canadian clades based on nucleotide sequence divergence.

According to the NJ phenogram for amino acid sequences, WBNP amino acid haplotypes belonging to clades a, b and c grouped with populations from Spain collected by Gomez et al. (2000b) (Figure 3-5). Average divergences of these clades from Spanish haplotypes were 0.48 to 0.97%, 0 to 0.48%, and 0.48 to 0.97% respectively. The WBNP amino acid haplotype belonging to clade d appeared paraphyletic to all other haplotypes from both Canada (WBNP) and Spain, with a range in amino acid divergence of 0 to 0.48% from other WBNP haplotypes and 0.48 to 0.97% from Spanish varieties.

3.3 Discussion

3.3.1 Between-Gene Comparisons

The 16S and COI analyses produced largely congruent phylogenies for congeneric taxa, but variations in tree topology occurred at the family and genus level. Past morphological studies have placed the genera *Keratella* and *Brachionus* in the family Brachionidae, while species of *Synchaeta* sp. are placed in the family Synchaetidae according to the structure of the mastax, which are muscles that activate a set of translucent sclerotized jaws known as the trophi (Pennak 1989). 16S and COI nucleotide neighbour-joining (NJ) (Appendix E and F) and 70% majority rule maximum parsimony (MP) tree topologies (Figure 3-3) were congruent with the exception that the COI MP tree did not resolve the two families according to morphological evidence. Additionally, COI varied from 16S in the placement of *Keratella hiemalis* in the NJ tree and in the positioning of freshwater *Brachionus* taxa in the MP cladogram. The COI nucleotide data were poor at resolving deep evolutionary relationships (e.g. families). Variation observed at the genus and species level between COI phenetic (Appendix F) and cladistic (Figure 3-3) trees was eliminated when nucleotide sequences were translated to amino acids (Appendix G and H), which produced tree topologies equivalent to those observed for 16S (Figure 3-2, Appendix E).

Similar levels of genetic divergence were apparent between the two genera of Brachionidae at both genes suggesting that the three genera belong to the same family (Tables 3-2 and 3-4). Alternatively, the similarity in nucleotide sequences between the Brachionidae and Synchaetidae may have been caused by homoplasy, in which multiple substitutions at nucleotide positions over time could have obscured differences (Page and

Holmes 1998). This was likely the case for COI, which evolves at a much faster rate than 16S (1.4 to 2.3% versus 0.4 to 0.9% per million years) (Cunningham et al. 1992, Sturmbauer et al. 1996, Knowlton and Weight 1998, Schubart et al. 1998). MP trees had higher homoplasy indices for COI (HI=0.47) compared to 16S rRNA (HI=0.28), and COI bootstrap values were low ($\leq 50\%$) for deep nodes between families and genera (Figure 3-3). It is possible that 18S rRNA, an even more slowly evolving nuclear gene (0.1% per million years) (Spears et al. 1992), would have been better for detecting familial boundaries than 16S rRNA. The more rapidly evolving COI gene, however, was most appropriate for resolution of recent evolutionary relationships at the population level.

3.3.2 *Phenotypic Plasticity Among Species*

Because environmental and genetic factors can cause morphological variability in rotifers, the clear establishment of species boundaries can be difficult (Pennak 1989). Both 16S and COI genes support the deep genetic divergence of *K. quadrata* from *K. hiemalis*. *K. quadrata* can be best identified by the large-bodied morph that has two long, curved posteriolateral spines. However, as a result of cyclomorphosis, this characteristic is highly variable and can cause confusion with the shorter-spined *K. hiemalis* (Ruttner-Kolisko 1993). *K. hiemalis* usually occurs at lower temperatures than *K. quadrata* (Stemberger 1979, Stemberger 1990, Ruttner-Kolisko 1993), and I have shown that the two species are genetically distinct. The common occurrence of interspecific hybridization in cladocerans with sequence divergences up to 14% in the 12S rRNA gene can complicate the resolution of species boundaries in co-occurring taxa

(Colbourne and Hebert 1996, Colbourne et al. 1998), but this phenomenon has not been reported in rotifers (Gomez et al. 2000b).

The taxonomy of *K. hiemalis* has been subject to considerable debate because some authors have reported the species as having low morphological variability in Europe (Ruttner-Kolisko 1993), while others have reported that the species shows considerable phenotypic plasticity in North America (Stemberger in the addendum for Ruttner-Kolisko 1993). *K. hiemalis* is easily confused with *K. testudo*, a common species in North American softwater lakes, because both species have a similar morphology and can have a reduction in one or both posteriolateral spines (Stemberger in Ruttner-Kolisko 1993, Stemberger 1979). I sequenced both typical two-spined *K. hiemalis* as well as its single-spined morph, but did not detect any genetic divergence between the forms. Both morphs were collected from hardwater lakes and were verified as *K. hiemalis* by the small spinules present on their lorica (R.S. Stemberger, pers. comm., Dartmouth College, New Hampshire). Whether *K. hiemalis* is one- or two-spined is likely determined by chemical cues exuded by predatory copepods and *Asplanchna* sp., which has been found to be the case for *K. testudo* (Stemberger and Gilbert 1987).

Keratella cochlearis has been recognized as a complex of different morphs that can vary in the degree of posterior spine development on their lorica (Dumont 1983). Long-spined individuals tend to dominate oligotrophic and cold habitats, while morphs with a reduction or absence of spines are abundant in eutrophic, warm environments (e.g. Hillbricht-Ilkowska 1983). Similar to *K. testudo*, induction of spine development in *K. cochlearis* is also affected by the presence of predators, and spined individuals are less susceptible to predation than unspined forms (Stemberger and Gilbert 1984, Conde-

Porcuna et al. 1993). Hofman's (1983) biometric analysis did not detect any transitional forms connecting spined and unspined morphs, which is suggestive of genetic divergence rather than phenotypic plasticity. The present COI nucleotide tree indicated that the *Keratella cochlearis* complex was comprised of two clades: a spined clade consisting of subspecies *K.c. robusta* and *K.c. faluta*, and an unspined clade comprised of *K.c. tecta*. The sequence divergence (4.4%) between spined and unspined taxa may explain variation for spine development in the *K. cochlearis* complex. A genetic basis for the ability to produce protective structures in response to chemical cues by predators has also been found in other freshwater zooplankton. Evolution of this trait in cladocerans is associated with adaptation to higher predation pressure during habitat shifts from ponds to lakes (Colbourne et al. 1997).

3.3.3 Genetic Divergence Within Species

Genetic divergence among *Brachionus plicatilis* populations that inhabit different saline lakes within a small geographic area was investigated with COI. The deep sequence divergence of saltwater *Brachionus* species from freshwater members of the genus collected elsewhere was supported by both 16S (8%) and COI (20%). Haplotypes of *B. plicatilis* clustered according to lakes from which they were collected in the COI nucleotide trees (Figure 3-3 and Appendix F). *B. plicatilis*-1, -2, and -3 were sampled from saline HC Lake and *B. plicatilis* -15 and -16 were collected from the more dilute subsaline FP Lake, which were only 10 km apart but did not share common streams (Fig. 3-1). The clade that includes haplotypes -4 to -14 was collected from two saline lakes that are interconnected by common streams (GB and SP lakes), and are 45 km away from HC and FP lakes (Figure 3-1). Studies of other inland zooplankton taxa have also found

little genetic structure among populations that inhabit interconnected lakes (De Meester 1996, Straughan and Lehman 2000). Clades belonging to HC, FP and interconnected GB/SP lakes had a mean sequence divergence of 0.7%, 0.9%, and 0.8%, respectively, from each other. This phylogenetic structuring of *B. plicatilis* haplotypes on a local scale likely reflects low dispersal and gene flow between populations (Gomez et al. 2000b) found in lakes that are not interconnected. Studies examining dispersal vectors, such as wind, rain, and waterfowl, have found that zooplankton dispersal is infrequent and limited to particular species (Jenkins and Underwood 1998). Experimental evidence for low dispersal of rotifers despite the high dispersal potential of resting eggs is contradictory because rotifers are among the first zooplankton to colonize new ponds (Jenkins 1995). However, genetic work has provided further evidence for restricted gene flow of passively dispersing aquatic organisms (e.g. DeMeester 1996, Colbourne et al. 1997, Gomez et al. 2000b).

Cyclically parthenogenetic populations, such as *Daphnia* sp., generally have little genetic divergence over thousands of kilometers (e.g. Weider et al. 1996, Weider et al. 1999) and even across possible barriers to dispersal, such as the Rocky mountains, in regions that were glaciated (Crease et al. 1997). By contrast, deep intraspecific genetic structure of passively dispersing zooplankton can be found over a broad geographic scale for *D. obtusa* (Hebert and Finston 1996), *D. laevis* (Taylor et al. 1998), and *Sida crystallina* (Cox and Hebert 2001) in unglaciated parts of North America where older lineages are in contact. Zooplankton populations appear to often be genetically structured on a local scale by evolutionary shifts between permanent (stable) and intermittent (unstable) habitats (De Meester 1996, Colbourne et al. 1997). The higher frequency of

extinction and recolonization events in ponds compared to longer-lived lakes may have a greater impact on mtDNA divergence than geographic distance (Crease et al. 1997).

Divergent selection between cyclic parthenogens inhabiting lakes and temporary ponds may occur because among other differences, lakes have relatively stable media and ponds undergo recurring periods of dessication that are thought to affect the amount and timing of sexual reproduction, as well as the population size (De Meester 1996). Lynch (1985) suggested that genetic drift is an important mechanism for population divergence in impermanent habitats because the onset of unfavourable conditions constrains the effective population size (Lynch 1985) and the small numbers of individuals that likely colonize new habitats may be subject to founder effects (Crease et al. 1997).

In contrast to haplotypes of *Brachionus plicatilis* which showed 0.3 to 1.3% divergence, freshwater *Keratella quadrata* showed much less COI nucleotide sequence divergence between populations within WBNP (0.2%). *K. quadrata* haplotypes inhabiting lakes within the 45 km radius in WBNP had similar sequence divergence to a population collected 330 km away (haplotype *K. quadrata*-5: mean of 0.5%). In addition, average nucleotide diversity (π) of *B. plicatilis* haplotypes was 8 times higher and the mean number of non-synonymous changes was more than twice as great as between *K. quadrata* haplotypes. The low intraspecific divergence of freshwater *K. quadrata* compared to halophilic *B. plicatilis* may be a result of inherent differences between the species, such as dispersal ability. Hatching of old resting eggs would decrease population divergence for both species, but this phenomenon is not understood (Serra et al. 1997). Additionally, freshwater lakes have relatively stable chemical environments compared to salt lakes, which can undergo dramatic fluctuations in salt concentration over a season

(Hammer 1986), and these spatial and temporal environmental gradients may influence population divergence.

Salinity fluctuations within FP Lake may have contributed to genetic divergence among *B. plicatilis* nucleotide haplotypes –15 and –16 (Figure 3-3, Appendix F and I), which correspond with the amino acid clade c (Figure 3-5, Appendix G and H). While these haplotypes were collected from a lake that fluctuates between saline and subsaline conditions (FP Lake), other haplotypes were sampled from lakes that remained saline throughout the season (HC, GB and SP lakes). Haplotypes occurring in lakes with relatively stable salinities likely underwent many asexual and sexual generations (Snell 1986). This may have resulted in slightly greater divergence from *B. plicatilis* –15 and –16, which likely persisted as dormant, sexually-produced resting eggs on the lake bottom as long as subsaline (840 mg/l total dissolved solids (TDS), summer mean) conditions prevailed. Another possibility is this population represented clones adapted to lower salinities (5000 mg/l TDS) than those found in the other three lakes (12 676 to 26 318 mg/l TDS, summer means). This is supported by the persistence of *B. plicatilis* in low densities throughout the summer in FP Lake, and the detection of saline water over the bottom sediments at the end of the summer (Chapter 2). The nucleotide and amino acid sequence divergences detected among the few *B. plicatilis* populations located within close proximity of each other in WBNP are slight and are not conclusive. Additionally, the magnitude of genetic divergence among *B. plicatilis* haplotypes indicates that the lineages last shared a common ancestor 300 000 to 700 000 years ago (Cunningham et al. 1992, Sturmbauer et al. 1996, Knowlton and Weight 1998, Schubart et al. 1998), but the lakes in which the lineages are found have existed for less than 8000

years (Smith 1994). However, it is possible that fluctuations between fresh and saline conditions within aquatic environments could exert similar evolutionary pressures as those hypothesized for intermittent ponds.

Phylogenetic comparisons of *B. plicatilis* haplotypes from Canada and Spain (Gomez et al. 2000b) revealed their clear genetic divergence (Figure 3-4, Appendix I), at 4.3% mean sequence divergence. Average sequence divergence between the two major Spanish lineages was 2.6%, which was much greater than observed in WBNP. However, within each of these clades, mean sequence divergence was 0.4% and 1.3%, which was similar to the distance between WBNP haplotypes. When nucleotide sequences were translated to amino acids, clades a, b (HC Lake), and c (FP Lake) clustered with Spanish haplotypes while clade d (GB/SP lakes) remained as a separate clade (Figure 3-5). This may be preliminary evidence for another sibling species in the *B. plicatilis* species complex as well as considerable structuring of the species within WBNP, Canada.

Although *B. plicatilis* has been recognized as two distinct taxonomic sibling species, *B. plicatilis* ss. and *B. rotundiformis* (Segers 1995), there is evidence for at least three sibling species on the Iberian Peninsula, Spain (Gomez et al. 1995, Ortells et al. 2000). Gomez et al. (1997) reported that these three species have different temperature and salinity preferences. A group of large-sized strains adapted to lower salinities (9000 mg/l TDS) belong to *B. plicatilis* ss. while a group of smaller strains adapted to higher salinities (13 000 mg/l TDS) belong to *B. rotundiformis*. A group of intermediate-sized strains adapted to both high and low salinities (9000 to 13 000 mg/l TDS) is a second clonal group of *B. rotundiformis* (Gomez et al. 1997). When strains from Russia, Austria, Australia, Japan, China, and Colorado, USA were included in an allozyme

analysis with the three Spanish sibling species, there was evidence for more sibling species and the *B. plicatilis*-*B. rotundiformis* division was clearly resolved among these nine populations. Further phylogenetic work on the *B. plicatilis* complex could reveal whether the WBNP haplotypes belong to *B. plicatilis* ss., *B. rotundiformis*, or whether they represent an unidentified sibling species.

The findings of this study supported my original hypotheses. The deep genetic divergence of spined and unspined clades within morphologically variable *Keratella cochlearis* indicates the likelihood that this taxon includes at least two species. Also supporting my predictions, there was a lack of genetic diversity detected among freshwater populations of *K. quadrata* compared to saltwater *Brachionus plicatilis* populations. However, this could be a result of inherent differences among different monogonont rotifer taxa or it could reflect accelerated evolution in the halophile. In agreement with my hypothesis, *B. plicatilis* appears to be genetically diverse across nearby saline lakes in northern Alberta, Canada. However, the mechanisms that cause population divergence are complex. In combination with stochastic events, adaptation to different lakewater salinities, connectivity of surface water among lakes, and fluctuation of salt concentration within lakes all likely facilitate population divergence. Preliminary evidence suggests that there is an additional sibling species from the salt lakes in WBNP in the *B. plicatilis* species complex. This study has shown that molecular phylogenetic studies are possible for monogonont rotifers, and future investigations will be able to address correlations between population divergence and lakewater salinity, as well as address the potential for new sibling species within *Keratella cochlearis* and *Brachionus*

plicatilis. Further application of molecular phylogenetics will improve our ability to address the diversity and distribution of rotifers.

Table 3-1. Number of haplotypes per rotifer species (haplotype names correspond with notation for COI sequences), number of replicate sequences per haplotype and locations of sample collection for 16S sequences.

Species	Haplotypes	Number of Sequences per Haplotype	Locations of Sample Collections
<i>Brachionus plicatilis</i>	<i>B. plicatilis</i> -2	1	WBNP (HC Lake)
	<i>B. plicatilis</i> -5	1	WBNP (GB Lake)
	<i>B.plicatilis</i> -11	3	WBNP (SP Lake)
<i>Brachionus calyciflorus</i>	<i>B.calyciflorus</i> -1	4	Gainesville, Florida
<i>Brachionus urceolaris</i>	<i>B. urceolaris</i> -1	1	Fort McMurray, AB
<i>Keratella quadrata</i>	<i>K. quadrata</i> -2	3	WBNP (WR Lake), Fort McMurray, AB
<i>Keratella hiemalis</i>	<i>K. hiemalis</i> -1	1	WBNP (BP Lake)
<i>Keratella cochlearis</i>	<i>K. cochlearis</i> -f1	1	WBNP (BP Lake)
	<i>K. cochlearis</i> -r1	2	Fort McMurray, AB
	<i>K. cochlearis</i> -t1	1	WBNP (FH Lake)
	<i>K. cochlearis</i> -t2	1	WBNP (FH Lake)
<i>Synchaeta</i> sp.	<i>S. c.f. pectinata</i> -1	2	WBNP (BP Lake)
	<i>S. c.f. pectinata</i> -2	3	WBNP (GW+FH Lakes)
	<i>Synchaeta</i> sp.-1	1	WBNP (BP Lake)
	<i>Synchaeta</i> sp.-2	1	WBNP (BP Lake)

Table 3-2. Mean decimal percent 16S divergence ± standard errors between phylogenetic clusters of rotifers identified by NJ (Appendix E).

Clade	B. calyciflorus	B. urceolaris	B. plicatilis	K. cochlearis	K. hiemalis	K. quadrata	Synchaeta sp.
B. calyciflorus	-	-	-	-	-	-	-
B. urceolaris	0.0406	-	-	-	-	-	-
B. plicatilis	0.0861± 9.7x10 ⁻⁷	0.0739± 9.3x10 ⁻⁷	0.0053± 2.3x10⁻⁶	-	-	-	-
K. cochlearis	0.2154± 9.3x10 ⁻⁶	0.2082± 3.3x10 ⁻⁶	0.2085± 1.8x10 ⁻⁶	0.0120± 2.3x10⁻⁶	-	-	-
K. hiemalis	0.2186	0.2257	0.2279± 1.4x10 ⁻⁶	0.1624± 2.8x10 ⁻⁶	-	-	-
K. quadrata	0.2191	0.2296	0.2406± 4.4x10 ⁻⁶	0.1842± 6.5x10 ⁻⁷	0.1067	-	-
Synchaeta sp.	0.1640± 2.0x10 ⁻⁶	0.1636± 1.4x10 ⁻⁵	0.1674± 1.8x10 ⁻⁶	0.2865± 4.4x10 ⁻⁶	0.2974± 9.2x10 ⁻⁵	0.3017± 7.0x10 ⁻⁵	0.0597± 1.4x10⁻⁴

Note: The mean nucleotide sequence divergence within clusters is shown on the diagonal (boldfaced). The mean nucleotide sequence divergence between clusters is shown below the diagonal. The distance estimates were corrected with Kimura’s (1980) two-parameter model.

Table 3-3. Number of haplotypes per rotifer species, number of individuals sequenced per haplotype, and locations of sample collection for COI sequences. *Brachionus plicatilis* was collected from saline waters > 3000 mg/l total dissolved solids (HC, GB, SP, and FP lakes). All other lakes were subsaline (500 to 3000 mg/l total dissolved solids).

Species	Haplotypes	Number of Sequences per Haplotype	Locations of Sample Collections
<i>Brachionus plicatilis</i>	<i>B. plicatilis</i> -1	1	WBNP (HC Lake)
	<i>B. plicatilis</i> -2	2	WBNP (HC Lake)
	<i>B. plicatilis</i> -3	1	WBNP (HC Lake)
	<i>B. plicatilis</i> -4	1	WBNP (GB Lake)
	<i>B. plicatilis</i> -5	1	WBNP (GB Lake)
	<i>B. plicatilis</i> -6	1	WBNP (GB Lake)
	<i>B. plicatilis</i> -7	1	WBNP (GB Lake)
	<i>B. plicatilis</i> -8	1	WBNP (GB Lake)
	<i>B. plicatilis</i> -9	1	WBNP (GB Lake)
	<i>B. plicatilis</i> -10	1	WBNP (GB Lake)
	<i>B. plicatilis</i> -11	4	WBNP (SP Lake)
	<i>B. plicatilis</i> -12	1	WBNP (SP Lake)
	<i>B. plicatilis</i> -13	1	WBNP (SP Lake)
	<i>B. plicatilis</i> -14	1	WBNP (SP Lake)
	<i>B. plicatilis</i> -15	2	WBNP (FP Lake)
	<i>B. plicatilis</i> -16	2	WBNP (FP Lake)
<i>Brachionus calyciflorus</i>	<i>B. calyciflorus</i> -1	2	Gainesville, Florida
<i>Brachionus urceolaris</i>	<i>B. urceolaris</i> -1	3	Fort McMurray, AB
	<i>B. urceolaris</i> -2	1	Fort McMurray, AB
	<i>B. urceolaris</i> -3	2	Fort McMurray, AB
	<i>B. urceolaris</i> -4	1	Fort McMurray, AB
<i>Keratella quadrata</i>	<i>K. quadrata</i> -1	3	WBNP (WR+FP Lakes)
	<i>K. quadrata</i> -2	3	WBNP (WR Lake)
	<i>K. quadrata</i> -3	7	WBNP (GL Lake)
	<i>K. quadrata</i> -4	1	WBNP (GW Lake)
	<i>K. quadrata</i> -5	8	Fort McMurray, AB
	<i>K. quadrata</i> -6	4	WBNP (FP+GW Lakes), Fort McMurray, AB
	<i>K. quadrata</i> -7	1	WBNP (WR Lake)
<i>Keratella hiemalis</i>	<i>K. hiemalis</i> -1	5	WBNP (BP+WR Lakes)
	<i>K. hiemalis</i> -2	1	WBNP (WR Lake)
	<i>K. hiemalis</i> -3	1	WBNP (WR Lake)
<i>Keratella cochlearis</i>	<i>K. cochlearis</i> -f1	1	WBNP (BP Lake)
	<i>K. cochlearis</i> -r1	1	Fort McMurray, AB
	<i>K. cochlearis</i> -r2	3	Fort McMurray, AB
	<i>K. cochlearis</i> -t1	2	WBNP (FH Lake)
	<i>K. cochlearis</i> -t2	2	WBNP (FH Lake)
<i>Synchaeta</i> sp.	<i>S. c.f. pectinata</i> -1	2	WBNP (BP Lake)
	<i>S. c.f. pectinata</i> -2	3	WBNP (FH Lake)
	<i>S. c.f. pectinata</i> -3	2	WBNP (GW Lake)
	<i>Synchaeta</i> sp.-4	1	WBNP (BP Lake)
	<i>Synchaeta</i> sp.-5	1	WBNP (BP Lake)
	<i>Synchaeta</i> sp. -6	3	WBNP (BP Lake)

Table 3-4. Mean decimal percent COI divergence \pm standard errors among rotifer species identified by NJ (Appendix F and G).

Clade	B. calyciflorus	B. urceolaris	B. plicatilis	K. cochlearis	K. hiemalis	K. quadrata	Synchaeta sp.
B. calyciflorus	-	0.0509	0.0379 \pm 2.2x10 ⁻⁵	0.1111	0.1111	0.0979 \pm 4.5x10 ⁻⁷	0.1250 \pm 1.8x10 ⁻⁵
B. urceolaris	0.2002 \pm 1.2x10 ⁻⁶	-	0.0550 \pm 4.1x10 ⁻⁸	0.1065	0.1157	0.0965 \pm 9.6x10 ⁻⁸	0.1356 \pm 3.6x10 ⁻⁶
B. plicatilis	0.2480 \pm 3.7x10 ⁻⁷	0.2562 \pm 1.2x10 ⁻⁷	0.0064\pm 5.5x10⁻⁸	0.1198 \pm 3.3x10 ⁻⁸	0.1213 \pm 7.3x10 ⁻⁸	0.1067 \pm 5.6x10 ⁻⁸	0.1353 \pm 3.3x10 ⁻⁷
K. cochlearis	0.2737 \pm 3.9x10 ⁻⁵	0.2874 \pm 5.0x10 ⁻⁷	0.3201 \pm 7.3x10 ⁻⁷	0.0277\pm 2.0x10⁻⁴	0.0833	0.0654 \pm 9.5x10 ⁻⁸	0.1192 \pm 1.7x10 ⁻⁶
K. hiemalis	0.2604 \pm 2.3x10 ⁻⁶	0.2692 \pm 5.3x10 ⁻⁷	0.3082 \pm 2.6x10 ⁻⁷	0.2559 \pm 7.5x10 ⁻⁷	0.0021\pm 2.8x10⁻⁷	0.0227 \pm 1.3x10 ⁻⁶	0.1396 \pm 5.6x10 ⁻⁶
K. quadrata	0.2823 \pm 2.1x10 ⁻⁶	0.2897 \pm 4.8x10 ⁻⁷	0.2789 \pm 1.0x10 ⁻⁷	0.2740 \pm 4.6x10 ⁻⁷	0.2371 \pm 4.2x10 ⁻⁷	0.0043\pm 1.5x10⁻⁷	0.1217 \pm 2.4x10 ⁻⁶
Synchaeta sp.	0.2453 \pm 1.0x10 ⁻⁴	0.2491 \pm 1.4x10 ⁻⁵	0.3270 \pm 9.9x10 ⁻⁷	0.3008 \pm 5.4x10 ⁻⁶	0.2920 \pm 3.5x10 ⁻⁶	0.3220 \pm 7.2x10 ⁻⁶	0.1328\pm 2.0x10⁻⁴

Note: The mean uncorrected (p distance) amino acid sequence divergence between clusters is shown above the diagonal and is based on mean character differences. The mean nucleotide sequence divergence within clusters is shown on the diagonal (boldfaced). The mean nucleotide sequence divergence between clusters is shown below the diagonal. The nucleotide distance estimates were corrected with the Tamura-Nei (1993) model.

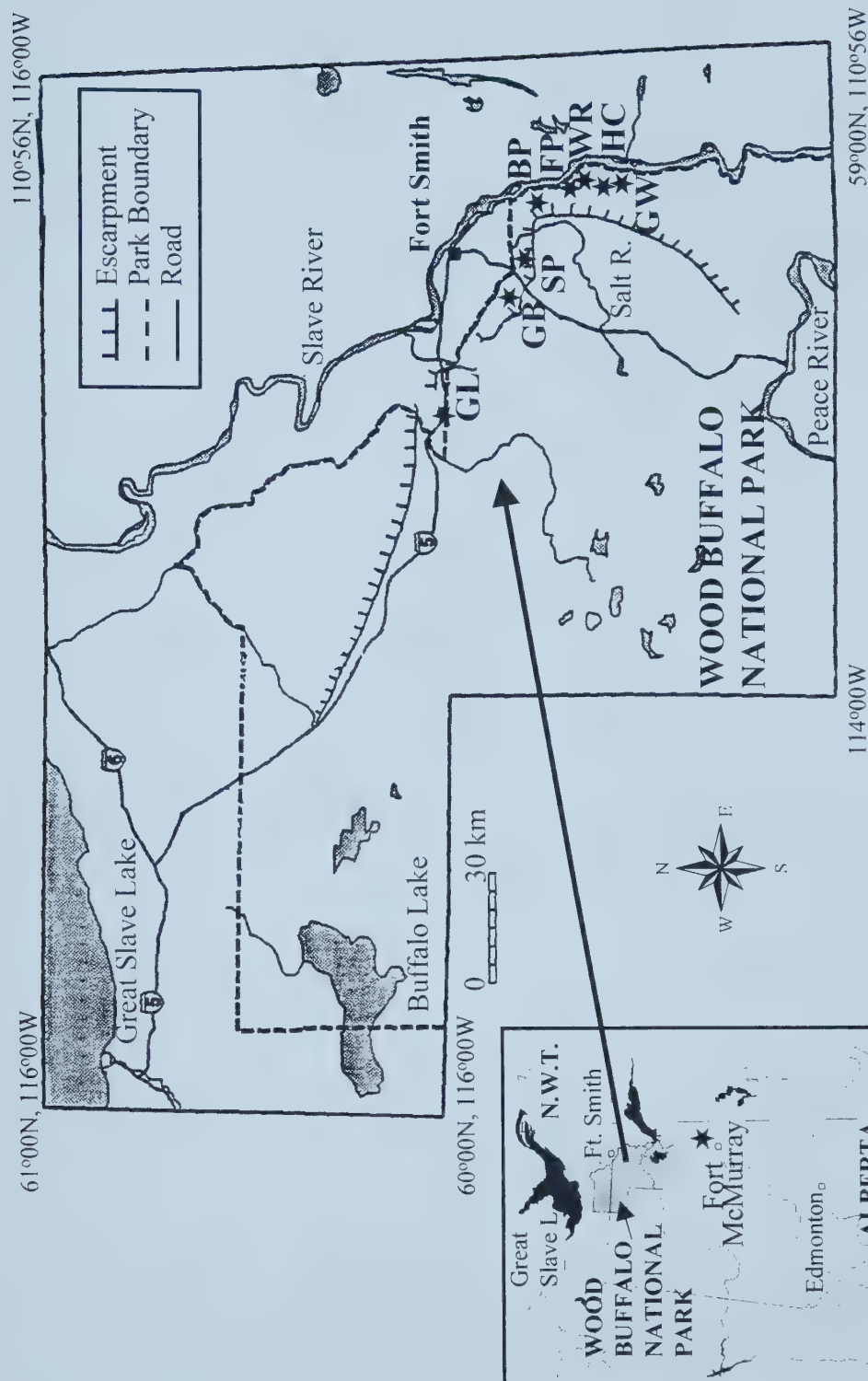


Figure 3-1. The location (★) of saline (GB, SP, FP, HC) and subsaline (GL, BP, WR, GW, and near Fort McMurray) lakes in Wood Buffalo National Park and Alberta, Canada from which rotifer DNA was sequenced.

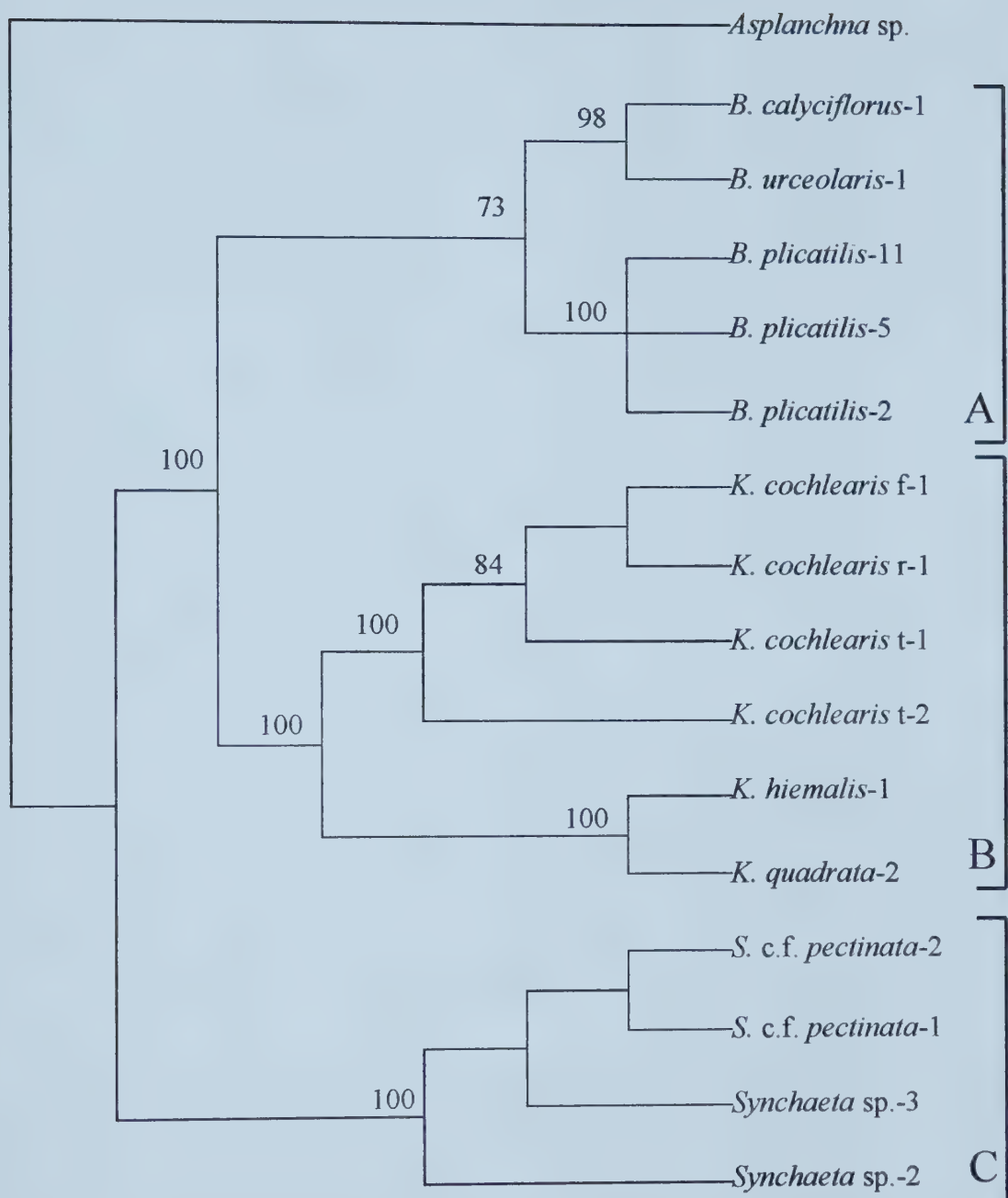


Figure 3-2. Seventy-percent majority rule concensus of 2 equally parsimonious trees for the 15 16S rDNA rotifer sequences rooted with *Asplanchna* sp. Bootstrap support (1000 pseudoreplicates) for major clades is indicated above the nodes. Numbers at the terminal branches give the haplotype and correspond with the numbering assigned to the same taxa for which COI sequences were obtained. Capitalized letter clades indicate clusters representing different genera: A = *Brachionus* sp., B = *Keratella* sp., and C = *Synchaeta* sp.

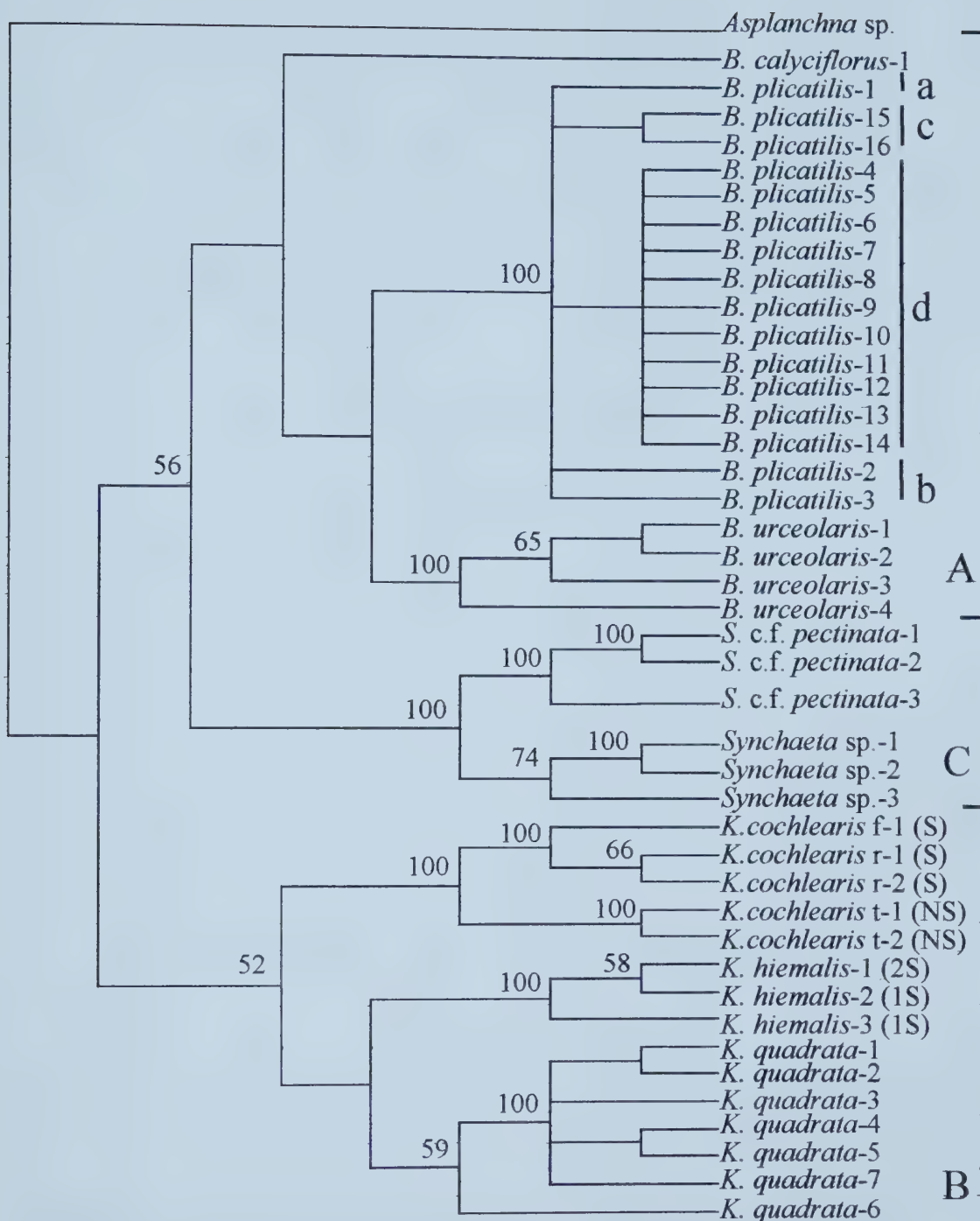


Figure 3-3. Seventy-percent majority rule concensus of 50 equally parsimonious trees of the 42 COI rotifer nucleotide sequences rooted with *Asplanchna* sp. Bootstrap support (1000 pseudoreplicates) for major clades is indicated above the nodes. Numbers at the terminal branches give the nucleotide haplotype and correspond with the numbering assigned to equivalent 16S taxa. Capitalized letter clades indicate clusters representing different genera: A = *Brachionus* sp., B = *Keratella* sp., and C = *Synchaeta* sp. Lowercase letter clades indicate clusters of *Brachionus plicatilis* haplotypes, which correspond to the lakes from which the animals were collected: clades a and b = HC Lake, clade c = FP Lake, and clade d = GL/SPL Lake. These clades also correspond with amino acid haplotypes in Appendix G. Spined (S) and unspined (NS) subspecies of *Keratella cochlearis* as well as two-spined (2S) and one-spined (1S) morphs of *K. hiemalis* are indicated on the figure.

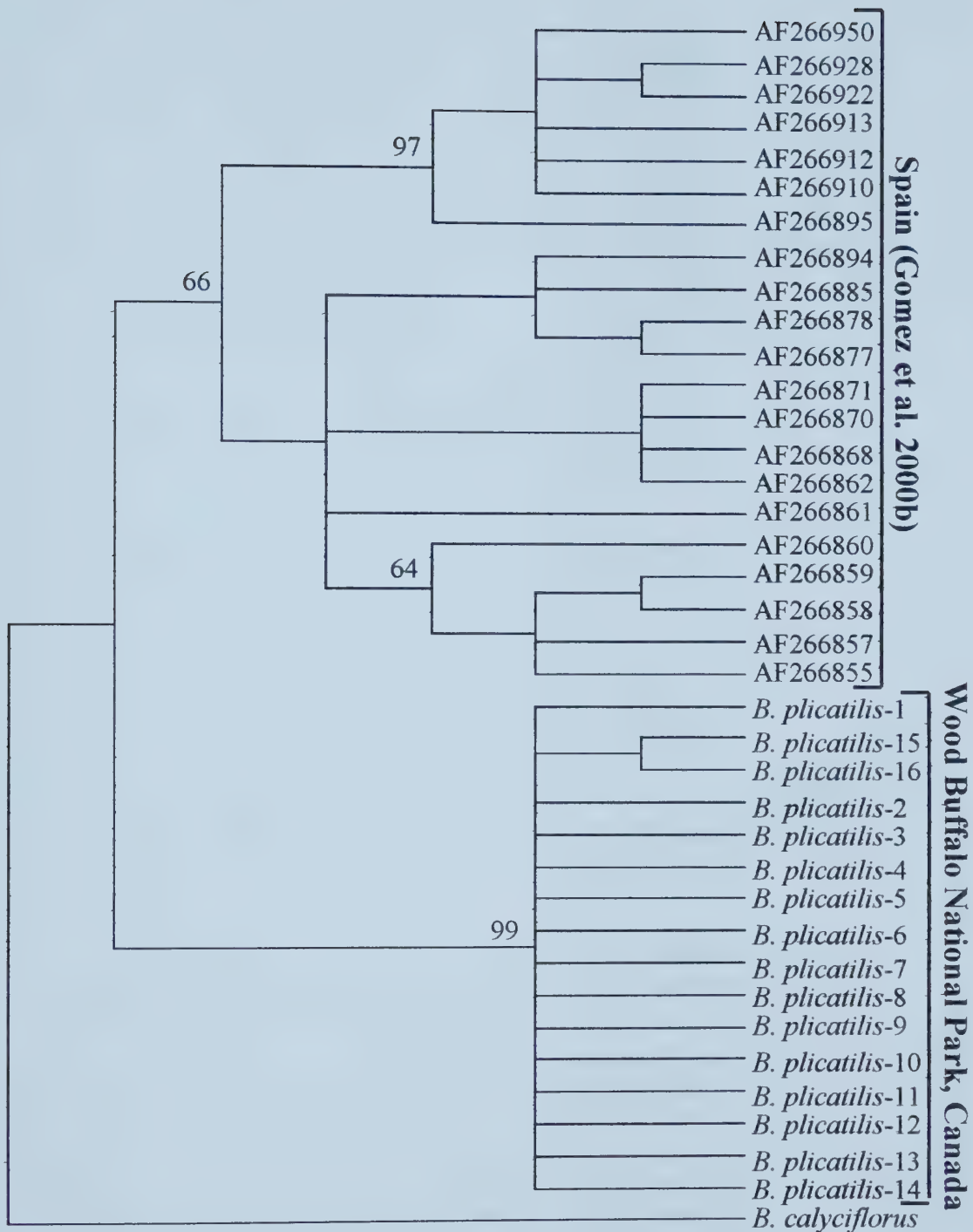


Figure 3-4. Seventy-percent majority rule consensus of 856 equally parsimonious trees of the 37 COI *Brachionus plicatilis* nucleotide sequences rooted with *B. calyciflorus*. Bootstrap support (1000 pseudoreplicates) for major clades is indicated above the nodes. Accession numbers AF266855 to AF266950 belong to the 21 haplotypes identified by Gomez et al. (2000b) in the Iberian Peninsula, Spain. *B. plicatilis* -1 to -16 are haplotypes found in Wood Buffalo National Park (WBNP), Canada. Numbers at the terminal branches give the nucleotide haplotype for WBNP populations and correspond with the numbering assigned on other 16S and COI trees.

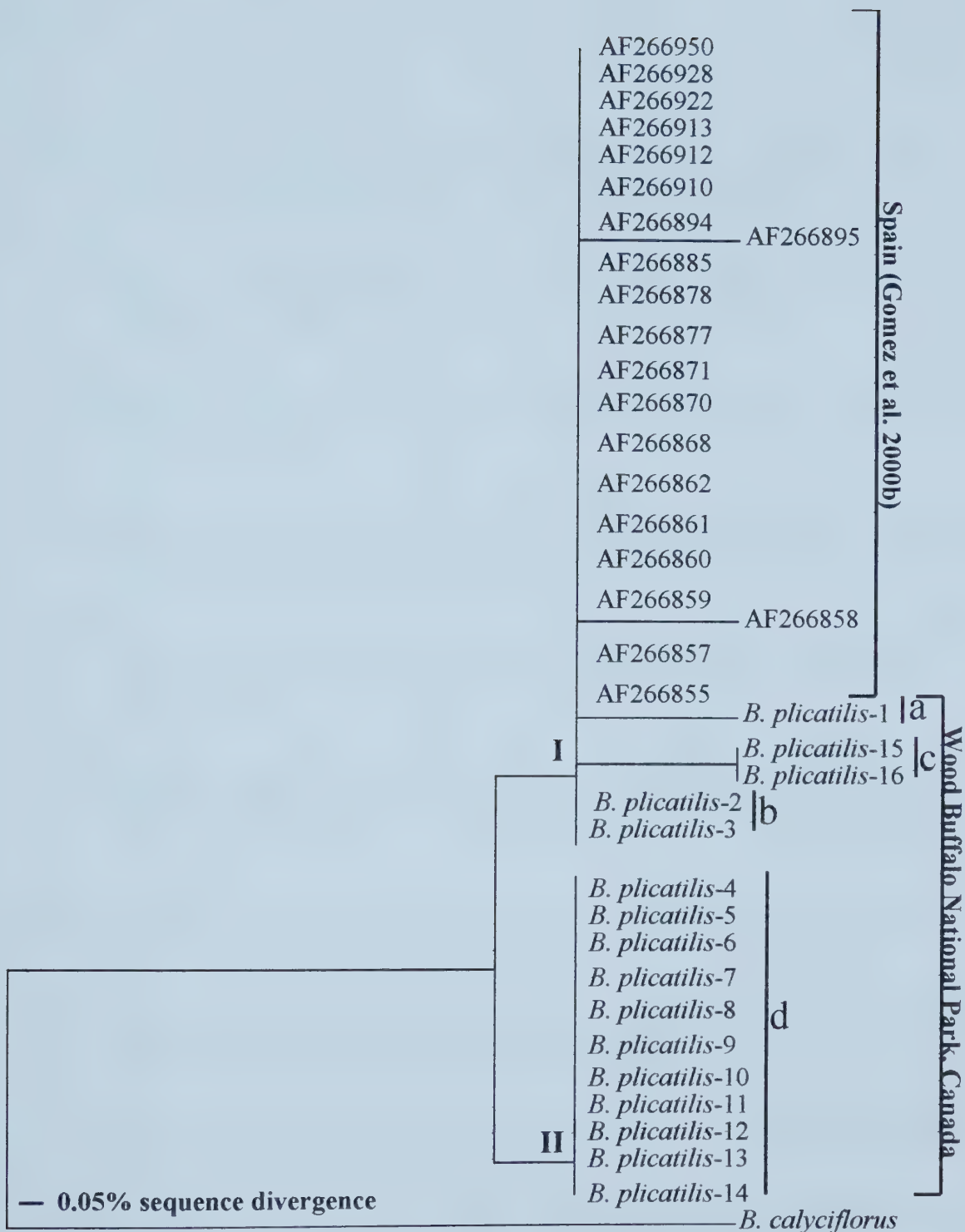


Figure 3-5. Neighbour-joining phenogram of 37 COI *Brachionus plicatilis* amino acid sequences based on uncorrected p-distances and rooting with *B. calyciflorus*. Accession numbers AF 266855 to AF266950 belong to 21 haplotypes identified by Gomez et al. (2000b) on the Iberian Peninsula, Spain. *B. plicatilis* -1 to -16 are haplotypes found in Wood Buffalo National Park (WBNP), Canada. Numbers at the terminal branches give nucleotide haplotypes for WBNP populations and correspond with the numbering assigned on other 16S and COI trees. Lowercase letter clades indicate clusters of WBNP *B. plicatilis* nucleotide haplotypes, which correspond to lakes from which the animals were collected: clades a and b = HC Lake, clade c = FP Lake, and clade d = GL/SPL Lake.

3.4 References:

- Altschul, S.F., T.L. Madden, A.A. Schaffer, J. Zhang, Z. Zhang, W. Miller and D.J. Lipman. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research* 25: 3389-3402.
- Archie, J.W. 1989. Homoplasy excess ratio: new indices for measuring levels of homoplasy in phylogenetic systematics and a critique of the consistency index. *Systematic Zoology* 38: 253-269.
- Conde-Porcuna, J.M., R. Morales-Baquero and L. Cruz-Pizarro. 1993. Effectiveness of the caudal spine as a defense mechanism in *Keratella cochlearis*. *Hydrobiologia* 255/256: 283-287.
- Colbourne, J.K., T.J. Crease, L.J. Weider, P.D.N. Hebert, F. Dufresne and A. Hobaek. 1998. Phylogenetics and evolution of a circumarctic species complex (Cladocera: *Daphnia pulex*). *Biological Journal of the Linnean Society* 65: 347-365.
- Colbourne, J.K. and P.D.N. Hebert. 1996. The systematics of North American *Daphnia* (Crustacea: Anomopoda): a molecular phylogenetic approach. *Philosophical Transactions of the Royal Society of London B* 351: 349-360.
- Colbourne, J.K., P.D.N. Hebert and D.J. Taylor. 1997. Evolutionary origins of phenotypic plasticity in *Daphnia*. In: T.J. Givnish and K.J. Sytsma (eds.). *Molecular Evolution and Adaptive Radiation*. Cambridge University Press, Cambridge. Pp. 163-188.
- Cox, A.J. and P.D.N. Hebert. 2001. Colonization, extinction, and phylogeographic patterning in a freshwater crustacean. *Molecular Ecology* 10: 371-386.
- Crease, T.J., S.K. Lee, S.L. Yu, K. Spitze, N. Lehman and M. Lynch. 1997. Allozyme and mtDNA variation in populations of the *Daphnia pulex* complex from both sides of the Rocky mountains. *Heredity* 79: 242-251.
- Cunningham, C.W., N.W. Blackstone and L.W. Buss. 1992. Evolution of king crabs from hermit crab ancestors. *Nature* 355: 539-542.
- De Meester, L. 1996. Local genetic differentiation and adaptation in freshwater zooplankton populations: patterns and processes. *Ecoscience* 3: 385-399.
- Dumont, H. 1983. Biogeography of rotifers. *Hydrobiologia* 104: 19-30.

- Folmer, O., M. Black, W. Hoeh, R. Lutz and R. Vrijenhoek. 1994. DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3: 294-299.
- Gilbert, D. 1992. *SeqApp version 1.9a. A multiple sequence editor for Macintosh computers*. Published electronically on the Internet at fly.bio.indiana.edu.
- Gomez, A., M.J. Carmona and M. Serra. 1997. Ecological factors affecting gene flow in the *Brachionus plicatilis* complex (Rotifera). *Oecologia* 111: 350-356.
- Gomez, A. and G.R. Carvalho. 2000a. Sex, parthenogenesis and genetic structure of rotifers: microsatellite analysis of contemporary and resting egg bank populations. *Molecular Ecology* 9: 203-214.
- Gomez, A., G.R. Carvalho and D.H. Lunt. 2000b. Phylogeography and regional endemism of a passively dispersing zooplankter: mitochondrial DNA variation in rotifer resting eggs. *Proceedings of the Royal Society of London* 267:2189-2197.
- Gomez, A. and M. Serra. 1995. Behavioural reproductive isolation among sympatric strains of *Brachionus* Muller 1786: insights into the status of this taxonomic species. *Hydrobiologia* 313/314: 111-119.
- Gomez, A., M. Temprano and M. Serra. 1995. Ecological genetics of a cyclical parthenogen in temporary habitats. *Journal of Evolutionary Biology* 8: 601-622.
- Gomez, A. and T.W. Snell. 1996. Sibling species and cryptic speciation in the *Brachionus plicatilis* species complex. *Journal of Evolutionary Biology* 9: 953-964.
- Hafner, M.S., P.D. Sudman, F.X. Villablanca, T.A. Spradling, J.W. Demastes and S.A. Nadler. 1994. Disparate rates of molecular evolution in cospeciating hosts and parasites. *Science* 265: 1087-1090.
- Hammer, U.T. 1986. *Saline lake ecosystems of the world*. Dr. W. Junk Publishers, Boston. 616 pp.
- Hartl, D.L. 2000. *A Primer of Population Genetics*, 3rd ed. Sinauer Associates, Sunderland, M.S. 221 pp.
- Hartl, D.L. and A.G. Clark. 1997. *Principles of Population Genetics*, 3rd ed. Sinauer Associates, Sunderland, M.A.
- Hebert, P.D.N. 1998. Variable environments and evolutionary diversification in inland waters. In: G.R. Carvalho (ed.). *Advances in Molecular Ecology*. IOS Press, Amsterdam. Pp. 267-290.

- Hebert, P.D.N. and T.L. Finston. 1996. Genetic differentiation in *Daphnia obtusa*: a continental perspective. *Freshwater Biology* 35:311-321.
- Hebert, P.D.N., E.A. Remigio, J.K. Colbourne, D.J. Taylor and C.C. Wilson. 2001. Accelerated molecular evolution in halophilic crustaceans. Submitted.
- Hillbricht-Ilkowska, A. 1983. Morphological variation of *Keratella cochlearis* (Gosse) in Lake Biwa, Japan. *Hydrobiologia* 104: 297-305.
- Hillis, D.M. and J.P. Heulsbeck. 1992. Signal, noise, and reliability in molecular phylogenetic analyses. *Journal of Heredity* 83: 189-195.
- Hillis, D.M. and J.J. Bull. 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Systematic Biology* 42: 182-192.
- Hofmann, W. 1983. On temporal variation in the rotifer *Keratella cochlearis* (Gosse): the question of 'Lauterborn-cycles'. *Hydrobiologia* 101: 247-254.
- Iwabe, N., K. Kuma and T. Takashi. 1996. Evolution of gene families and relationship with organismal evolution: rapid divergence of tissue-specific genes in the early evolution of chordates. *Molecular Biology and Evolution* 13: 483-493.
- Jenkins, D.G. 1995. Dispersal-limited zooplankton distribution and community composition in new ponds. *Hydrobiologia* 313/314: 15-20.
- Jenkins, D.G. and M.O. Underwood. 1998. Zooplankton may not disperse readily in wind, rain, or waterfowl. *Hydrobiologia* 387/388: 15-21.
- Kerfoot, W.C. 1980. Perspectives on cyclomorphosis: separation of phenotypes and genotypes. In: Kerfoot, W.C. (ed.). *Evolution and Ecology of Zooplankton Communities*. University Press of New England, Hanover, N.H. Pp. 470-496.
- Kimura, M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* 16: 111-120.
- King, C.E. 1980. The genetic structure of zooplankton populations. In: W.C. Kerfoot (ed.). *Evolution and Ecology of Zooplankton Communities*. University Press of New England, Hanover, N.H. Pp. 315-328.
- Knowlton, N. and L.A. Weight. 1998. New dates and new rates for divergence across the Isthmus of Panama. *Proceedings of the Royal Society of London* 265: 2257-2263.

- Kumar, S., K. Tamura and S. Nei. 1993. MEGA: Molecular Evolutionary Genetic Analysis version 1.02. Pennsylvania State University, University Park, Pa.
- Lynch, M. 1985. Speciation in the Cladocera. *Verhandlungen Internationale Vereinigung für Limnologie* 22: 3116-3123.
- MacIsaac, H.J., T.C. Hutchinson and W. Keller. 1987. Analysis of planktonic rotifer assemblages from Sudbury, Ontario, area lakes of varying chemical composition. *Canadian Journal of Fisheries and Aquatic Sciences* 44: 1692-1701.
- Maley, L.E. and C.R. Marshall. 1998. The coming of age of molecular systematics. *Science* 279: 505-506.
- Miracle, M.R. and M. Serra. 1989. Salinity and temperature influence in rotifer life history characteristics. *Hydrobiologia* 186/187: 81-102.
- Ortells, R., T.W. Snell, A. Gomez and M. Serra. 2000. Patterns of genetic differentiation in resting egg banks of a rotifer species complex in Spain. *Archiv für Hydrobiologie* 149: 529-551.
- Palumbi, S.R. 1996. Nucleic acids II: the polymerase chain reaction. In: D.M. Hillis, C. Moritz and B.K. Mable (eds.). *Molecular Systematics*, 2nd ed. Sinauer Associates Inc., Sunderland, MA. Pp. 205-248.
- Page, R.D.M. and E.C. Holmes. 1998. *Molecular evolution: a phylogenetic approach*. Blackwell Science Ltd, Oxford. 346 pp.
- Pennak, R.W. 1989. Rotifera (rotifers). In: R.W. Pennak (ed.). *Fresh-water invertebrates of the United States: Protozoa to Mollusca*, 3rd ed. John Wiley & Sons Inc., New York. Pp. 169-225.
- Reed, R.D. and F.A.H. Sperling. 1999. Interaction of process partitions in phylogenetic analysis: an example from the swallowtail butterfly genus *Papilio*. *Molecular Biology and Evolution* 16: 286-297.
- Ruttner-Kolisko, A. 1993. Taxonomic problems with the species *Keratella hiemalis*. *Hydrobiologia* 255/256: 441-443.
- Saiki, R.K., D.H. Gelfand and S. Stoffel. 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* 239: 487-491.
- Schubart, C.D., R. Diesel, and S.B. Hedges. 1998. Rapid evolution to terrestrial life in Jamaican crabs. *Nature* 393: 363-365.

- Schwenk, K. 1996. Evolutionary genetics of *Daphnia* species complex: hybridism in syntopy. Publication 2231, Netherlands Institute of Ecology, Centre for Limnology, Nieuwersluis, The Netherlands.
- Segers, H. 1995. Nomenclatural consequences of some recent studies on *Brachionus plicatilis* (Rotifera: Brachionidae). *Hydrobiologia* 313/314: 121-122.
- Serra, M., A. Galiana and A. Gomez. 1997. Speciation in monogonont rotifers. *Hydrobiologia* 358: 63-70.
- Serra, M., A. Gomez and M.J. Carmona. 1998. Ecological genetics of *Brachionus* sibling species. *Hydrobiologia* 387/388: 373-384.
- Slatkin, M. 1985. Gene flow in natural populations. *Annual Review of Ecology and Systematics* 16: 393-430.
- Smith, D.G. 1994. Glacial Lake McConnell: paleogeography, age, duration, and associated river deltas, Mackenzie River Basin, Western Canada. *Quaternary Science Reviews* 13: 829-843.
- Snell, T.W. 1980. Blue-green algae and selection in rotifer populations. *Oecologia* 46: 343-346.
- Snell, T.W. 1986. Effect of temperature, salinity and food level on sexual and asexual reproduction in *Brachionus plicatilis* (Rotifera). *Marine Biology* 92: 157-162.
- Snell, T.W., B.D. Moffat, C. Janssen and G. Persoone. 1991a. Acute toxicity tests using rotifers. III. Effects of temperature, strain, and exposure time on the sensitivity of *Brachionus plicatilis*. *Environmental Toxicology and Water Quality* 6: 63-75.
- Snell, T.W., B.D. Moffat, C. Janssen and G. Persoone. 1991b. Acute toxicity tests using rotifers. IV. The effects of cyst age, temperature and salinity on the sensitivity of *Brachionus calyciflorus*. *Ecotoxicology and Environmental Safety* 21: 308-317.
- Spears, T., L.G. Abele and W. Kim. 1992. The monophyly of brachyuran crabs: a phylogenetic study based on 18S rRNA. *Systematic Biology* 41: 446-461.
- Stemberger, R.S. 1979. A Guide to Rotifers of the Laurentian Great Lakes. U.S. Environmental Protection Agency, Cincinnati, Ohio. (Available from National Technical Information Service, Springfield, Virginia. PB80-101280). 185 pp.
- Stemberger, R.S. and J.J. Gilbert. 1987. Multiple-species induction of morphological defenses in the rotifer *Keratella testudo*. *Ecology* 68: 370-378.

- Sternberger, R.S. and J.J. Gilbert. 1984. Spine development in the rotifer *Keratella cochlearis*: induction by cyclopoid copepods and *Asplanchna*. *Freshwater Biology* 14: 639-647.
- Straughan, D.J. and N. Lehman. 2000. Genetic differentiation among Oregon lake populations of the *Daphnia pulex* species complex. *Journal of Heredity* 91:8-17.
- Sturmbauer, Ch., J.S. Levinton, and J. Christy. 1996. Molecular phylogeny analysis of fiddler crabs: test of the hypothesis of increasing behavioral complexity in evolution. *Proceedings of the National Academy of Sciences USA* 93: 10855-10857.
- Swofford, D.L. 1998. PAUP*: Phylogenetic analysis using parsimony Version 4.0b2. Sinauer Associates, Sunderland M.A.
- Swofford, D.L., G.J. Olsen, P.J. Waddell and D.M. Hillis. 1996. Phylogenetic inference. In: Hillis, D.M, C. Moritz and B.K. Mable (eds.). *Molecular Systematics*, 2nd ed. Sinauer Associates Inc., Sunderland, MA. Pp. 407-514.
- Tamura, K. and M. Nei. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution* 10: 512-526.
- Taylor, D.J., T.L. Finston and P.D.N. Hebert. 1998. Biogeography of a widespread freshwater crustacean: pseudocongruence and cryptic endemism in the North American *Daphnia laevis* complex. *Evolution* 52: 1648-1670.
- Wallace, R.L. and T.W. Snell. 1991. Rotifera. In: Thorpe, J.H. and A.P. Covich (eds.). *Ecology and Classification of North American Invertebrates*. Academic Press, Inc., Harcourt Brace Jovanovich Publishers, San Diego. Pp. 187-248.
- Walsh, E.J. 1993. Rotifer genetics: integration of classic and modern techniques. *Hydrobiologia* 255/256: 193-204.
- Weider, L.J. and P.D.N. Hebert. 1987. Ecological and physiological differentiation among low-arctic clones of *Daphnia pulex*. *Ecology* 68: 188-198.
- Weider, L.J., A. Hobaek, T.J. Crease and H. Stibor. 1996. Molecular characterization of clonal population structure and biogeography of arctic apomictic *Daphnia* from Greenland and Iceland. *Molecular Ecology* 5: 107-118.
- Weider, L.J., A. Hobaek, J.K. Colbourne, T.J. Crease, F. Dufresne and P.D.N. Hebert. 1999. Holarctic phylogeography of an asexual species complex I. MtDNA variation in arctic *Daphnia*. *Evolution* 53: 777-792.

- Wilson, C.C. and P.D.N. Hebert. 1992. The maintenance of taxon diversity in an asexual assemblage: an experimental analysis. *Ecology* 73: 1462-1472.
- Wuyts, J., P. De Rijk, Y. Van de Peer, T. Winkelmans and R. De Wachter. 2001. The European Ribosomal RNA database. *Nucleic Acids Research* 29:175-177.
- Xia, X., M.S. Hafner and P.D. Sudman. 1996. On transition bias in mitochondrial genes of pocket gophers. *Journal of Molecular Evolution* 43: 32-40.

CHAPTER 4: GENERAL CONCLUSIONS

4.0 General Conclusions

It is generally agreed that increased lakewater salinity results in reduced aquatic biodiversity (Hammer 1990, Williams et al. 1990, Frey 1993, Hammer 1993, Williams 1993). However, little is known about how different types of salts affect the composition of zooplankton communities (Frey 1993, Bos et al. 1996, Williams 1998, Bos et al. 1999). I found that two lakewater ion types, chloride-dominated and sulphate/carbonate-dominated, had distinct communities of zooplankton. While saline lakes containing water dominated by chloride anions had an abundance of the rotifers *Brachionus plicatilis* and *Hexarthra* sp., and the harpacticoid copepod *Cletocamptus* sp., sulphate/carbonate-dominated lake water was characterized by the prevalence of the crustaceans, *Leptodiaptomus sicilis*, *Diaptomus nevadensis*, and *Daphnia similis*. *L. sicilis* was positively associated with elevated sulphate concentrations in both saline and more dilute subsaline lake water. However, other differences in zooplankton community composition between the two lakewater ion types could not entirely be attributed to differences in water chemistry. Top-down predation by nine-spined stickleback (*Pungitius pungitius*) (Nelson and Paetz 1992) may have eliminated larger crustacean zooplankton in the lakes with chloride-dominated water. Cladocerans and calanoid copepods were more abundant in the fishless lakes containing water dominated by sulphate/carbonate anions. My study suggests that both salt ion composition and different predation regimes are important in determining zooplankton communities in saline aquatic environments.

In addition to changes in zooplankton communities that may occur at different salinities and with different salts, gradients in salinity may contribute to evolutionary diversification within salt-tolerant species (e.g. Gomez et al. 1995). Rotifers are an understudied component of zooplankton that frequent salt lakes (Hammer 1993). These animals are a challenge to identify because of their small size (most are 200 to 500 μm long) (Pennak 1989), phenotypic plasticity (Serra et al. 1998), cyclic parthenogenic mode of reproduction, and potential for cryptic speciation in the absence of morphological change (Serra et al. 1997). My study is among the first molecular phylogenetic work conducted on rotifers, which has recently become possible through technical advances in the polymerase chain reaction. I found that spined and unspined clades of *Keratella cochlearis* represent at least two species and in combination with the results of Gomez et al. (2000), provide preliminary evidence for an additional sibling species within the *Brachionus plicatilis* species complex. Greater haplotype diversity and divergence was observed among populations of halophilic *Brachionus plicatilis* than among populations of predominantly freshwater *Keratella quadrata*. The most divergent *B. plicatilis* population was a strain that was likely adapted to lower salinity than the other haplotypes. Fluctuations in lakewater salt concentrations that spanned osmoregulatory tolerance limits for *B. plicatilis* may have created conditions that contributed to the greater divergence of this population. Because evolutionary change is slow and occurs over millions of years, it is difficult to relate this phenomenon to changes in the environment caused by relatively recent changes (Travis and Reznick 1998). However, it is possible that salinity may reduce the diversity of zooplankton communities and yet also contribute

to the conditions that, with time, allow for genetic diversification among halophilic zooplankton populations.

4.1 References

- Bos, D.G., B.F. Cumming and J.P. Smol. 1999. Cladocera and Anostraca from the Interior Plateau of British Columbia, Canada, as paleolimnological indicators of salinity and lake level. *Hydrobiologia* 392: 129-141.
- Bos, D.G., B.F. Cumming, C.E. Watters and J.P. Smol. 1996. The relationship between zooplankton, conductivity and lake-water ionic composition in 111 lakes from the Interior Plateau of British Columbia, Canada. *International Journal of Salt Lake Research* 5: 1-15.
- Frey, D.G. 1993. The penetration of cladocerans into saline waters. *Hydrobiologia* 267: 233-248.
- Gomez, A., G.R. Carvalho and D.H. Lunt. 2000. Phylogeography and regional endemism of a passively dispersing zooplankton: mitochondrial DNA variation in rotifer resting eggs. *Proceedings of the Royal Society of London* 267: 2189-2197.
- Gomez, A., M. Temprano and M. Serra. 1995. Ecological genetics of a cyclical parthenogen in temporary habitats. *Journal of Evolutionary Biology* 8: 601-622.
- Hammer, U.T. 1990. The effects of climate change on the salinity, water levels and biota of Canadian prairie saline lakes. *Verhandlungen Internationale Vereinigung für Limnologie* 24: 321-326.
- Hammer, U.T. 1993. Zooplankton distribution and abundance in saline lakes of Alberta and Saskatchewan, Canada. *International Journal for Salt Lake Research* 2: 111-132.
- Nelson, J.S. and M.J. Paetz. 1992. *The fishes of Alberta*. University of Alberta Press, Edmonton. 437 pp.
- Pennak, R.W. 1989. Rotifera (rotifers). In: R.W. Pennak (ed.). *Fresh-water invertebrates of the United States: Protozoa to Mollusca*, 3rd ed. John Wiley & Sons Inc., New York. Pp. 169-225.
- Serra, M., A. Galiana and A. Gomez. 1997. Speciation in monogonont rotifers. *Hydrobiologia* 358: 63-70.
- Serra, M., A. Gomez and M.J. Carmona. 1998. Ecological genetics of *Brachionus* sibling species. *Hydrobiologia* 387/388: 373-384.
- Travis, J. and D.N. Reznick. 1998. Experimental approaches to the study of evolution. In: Resetarits Jr., W.J. and J. Bernardo (eds.). *Experimental Ecology: Issues and Perspectives*. Oxford University Press, New York. Pp. 437-459.

- Williams, W.D. 1993. The worldwide occurrence and limnological significance of falling water-levels in large, permanent saline lakes. *Verhandlungen Internationale Vereinigung für Limnologie* 25: 980-983.
- Williams, W.D. 1998. Salinity as a determinant of the structure of biological communities in salt lakes. *Hydrobiologia* 381: 191-201.
- Williams, W.D., A.J. Boulton and R.G. Taaffe. 1990. Salinity as a determinant of salt lake fauna: a question of scale. *Hydrobiologia* 197: 257-266.

APPENDIX A. List of rotifer families and taxa that are likely to be encountered in North America. This does not include rare freshwater genera that have been found on other continents and may eventually be found in North America. Numbers of species are approximations for the numbers reported in North America (Pennak 1989, Wallace and Snell 1991). Bolded taxa were subject to phylogenetic analysis in Chapter 3.

Phylum Rotifera (2000 recognized species)

Class Digononta

Order Seisonidea (2 recognized species)

Seison sp.

Order Bdelloidea (380 recognized species)

Family Philodinidae

Dissotrocha sp., *Embata* sp., *Macrotrachela* sp.,
Mniobia sp., *Philodina* sp., *Pleuretra* sp., *Rotaria* sp.

Family Habrotrochidae

Ceratotrocha sp., *Habrotrocha* sp., *Scepanotrocha* sp.

Family Philodinavidae

Philodinavus sp.

Family Adinetidae

Adineta sp., *Bradysclea* sp.

Class Monogononta (>1600 recognized species)

Order Flosculariacea

Family Flosculariidae

Beauchampia sp., *Floscularia* sp., *Lacinularia* sp.,
Limnias sp., *Octotrocha* sp., *Pseudoecistes* sp., *Ptygura* sp.,
Sinantherina sp.

Family Conochilidae

Conochilus sp., *Conochiloides* sp.

Family Hexarthridae

Hexarthra sp.

APPENDIX A (continued)

Family Tesudinellidae

Pompholyx sp., *Testudinella* sp.

Family Filiniidae

Filinia sp., *Tetramastix* sp.

Family Trochosphaeridae

Trochosphaera sp.

Order Collothecacea

Family Collothecidae

Collotheca sp., *Stephanoceros* sp.

Family Atrochidae

Acychus sp., *Atrochus* sp., *Cupelopagis* sp.

Order Ploima

Family Notommatidae

Cephalodella sp., *Drilophaga* sp., *Enteroplea* sp.,
Eosphora sp., *Eothinia* sp., *Itura* sp., *Monommata* sp.,
Notommata sp., *Pleurotrocha* sp., *Resticula* sp.,
Rousseletia sp., *Scaridium* sp., *Sphyrias* sp.,
Taphrocampa sp., *Tylotrocha* sp.

Family Tetrasiphoninae

Tetrasiphon sp.

Family Proalidae

Brycella sp., *Proales* sp., *Proalinopsis* sp.

Family Lindiidae

Lindia sp.

Family Birgeidae

Birgea sp.

Family Dicranophoridae

Albertia sp., *Aspelta* sp., *Dicranophorus* sp., *Dorria* sp.,
Encentrum sp., *Erignatha* sp., *Myersinella* sp.,
Pedipartia sp., *Streptognatha* sp., *Wierzejskiella* sp.

APPENDIX A (continued)

Order Ploima (continued)

Family Synchaetidae

Polyarthra sp., *Ploesoma* sp., *Pseudoploesoma* sp.,
***Synchaeta* sp. (>12 species)**

Family Microcodonidae

Microcodon sp.

Family Gastropodidae

Ascomorpha sp., *Chromogaster* sp., *Gastropus* sp.

Family Trichocercidae

Ascomorphella sp., *Elosa* sp., *Trichocerca* sp.

Family Asplanchidae

Asplanchna sp., *Asplanchnopus* sp., *Harringia* sp.

Family Epiphanidae

Cyrtonia sp., *Epiphanes* sp., *Mikrocodides* sp.,
Rhinoglena sp.

Family Brachionidae

Anuraeopsis sp., *Argonotholca* sp., *Kellicottia* sp.,
Notholca sp., *Platytias* sp., ***Brachionus* sp. (25 species),**
***Keratella* sp. (>15 species).**

Family Euchlanidae

Beauchampiella sp., *Dipleuchlanis* sp., *Euchlanis* sp.,
Tripleuchlanis sp.

Family Mytilinidae

Lophocharis sp., *Mytilina* sp.

Family Trichotriidae

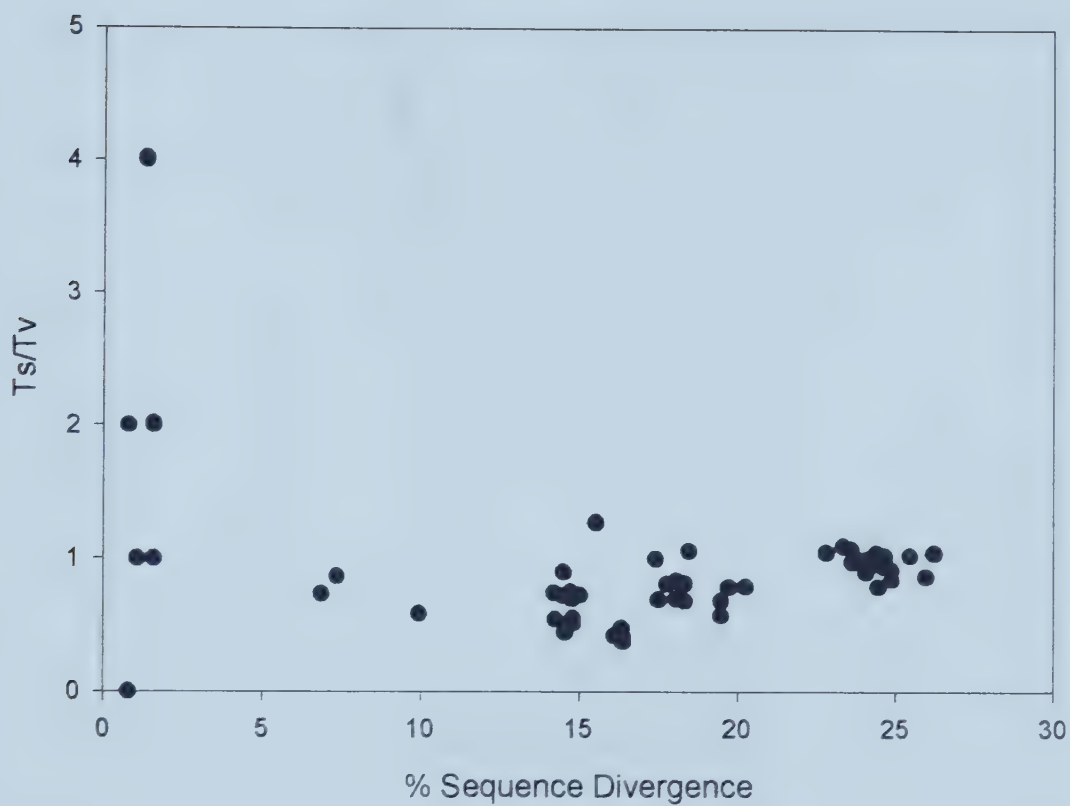
Macrochaetus sp., *Trichotria* sp.

Family Colurellidae

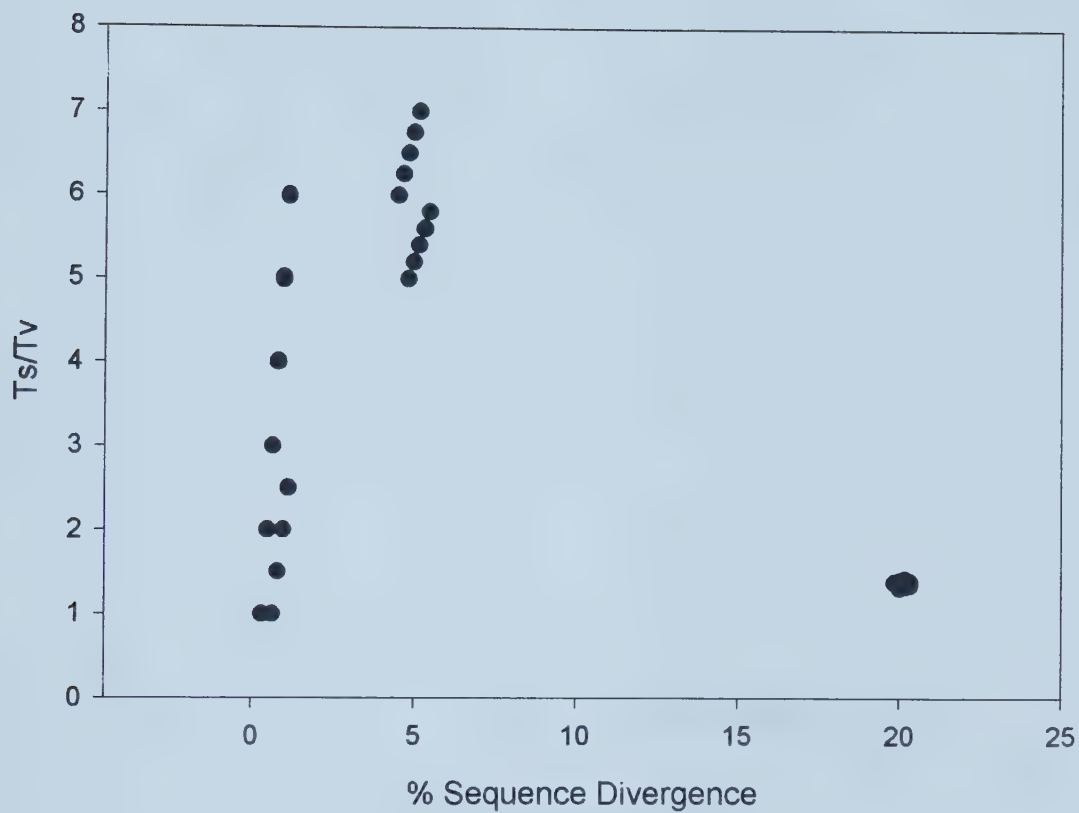
Colurella sp., *Lepadella* sp., *Paracolurella* sp.,
Squatinella sp.

Family Lecanidae

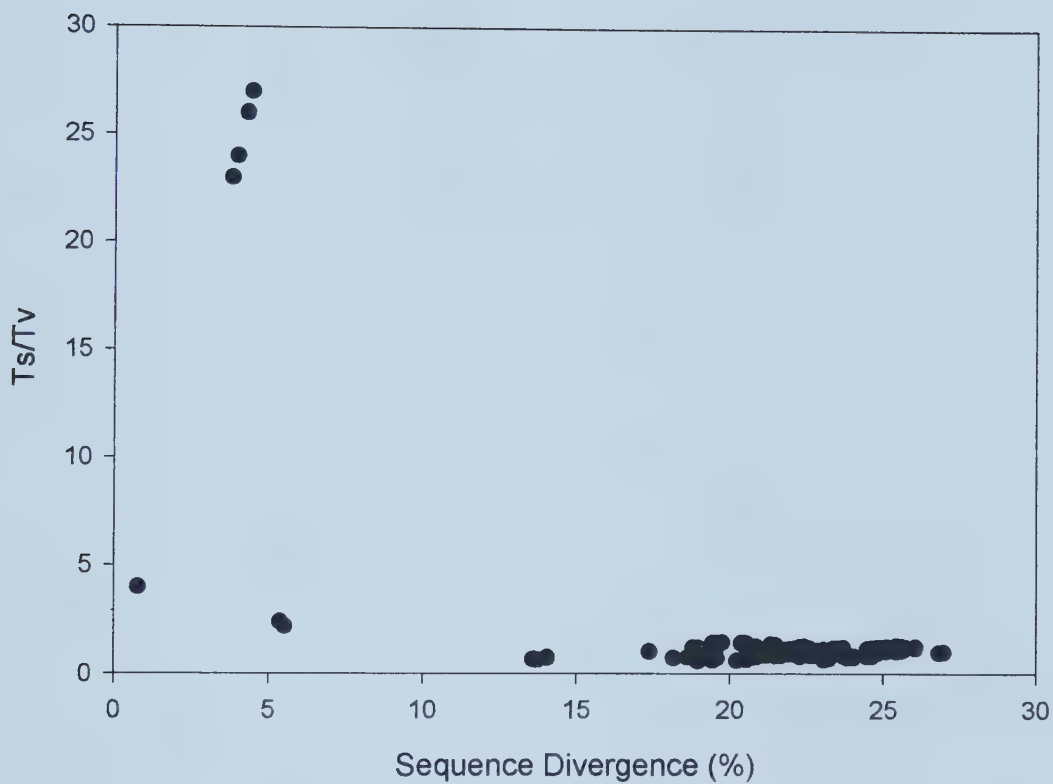
Lecane sp.



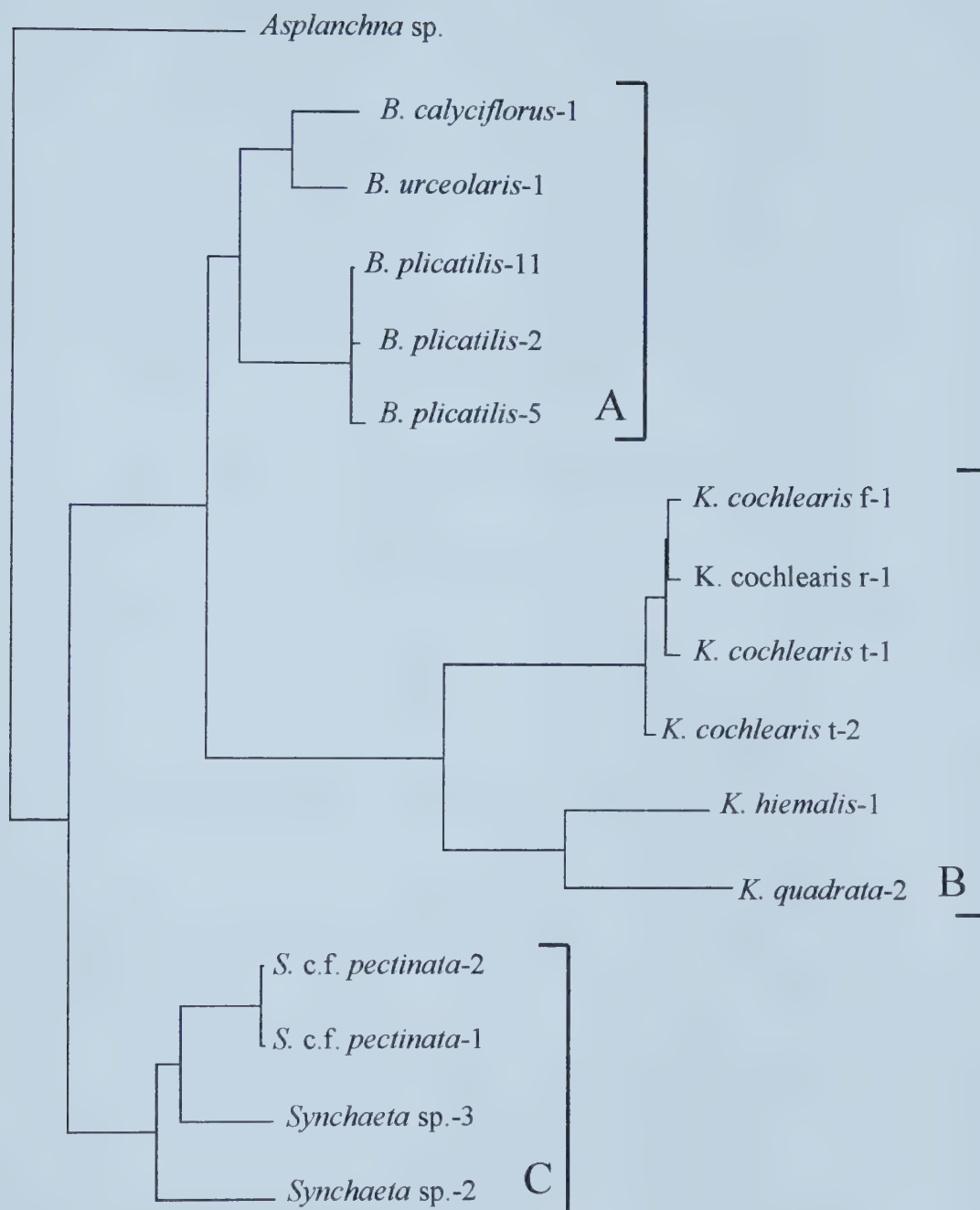
Appendix B. Scatter plot of transition/transversion (Ts/Tv) ratios versus percent sequence divergence for pairwise comparisons of 16S sequences isolated from rotifer species.



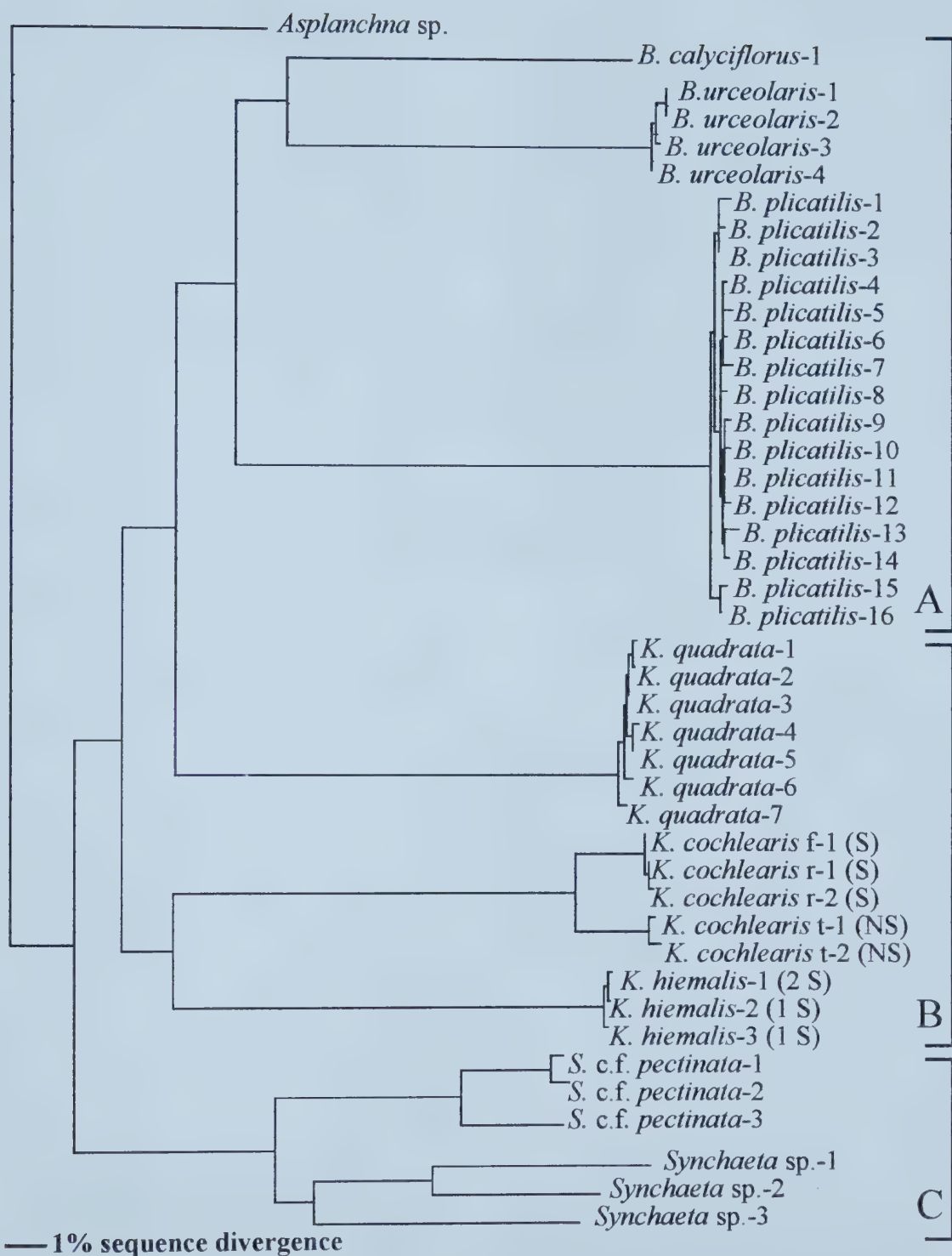
Appendix C. Scatter plot of transition/transversion (Ts/Tv) ratios versus percent sequence divergence for pairwise comparisons of *Brachionus plicatilis* haplotypes sequenced for this study and reported by Gomez et al. (2000b).



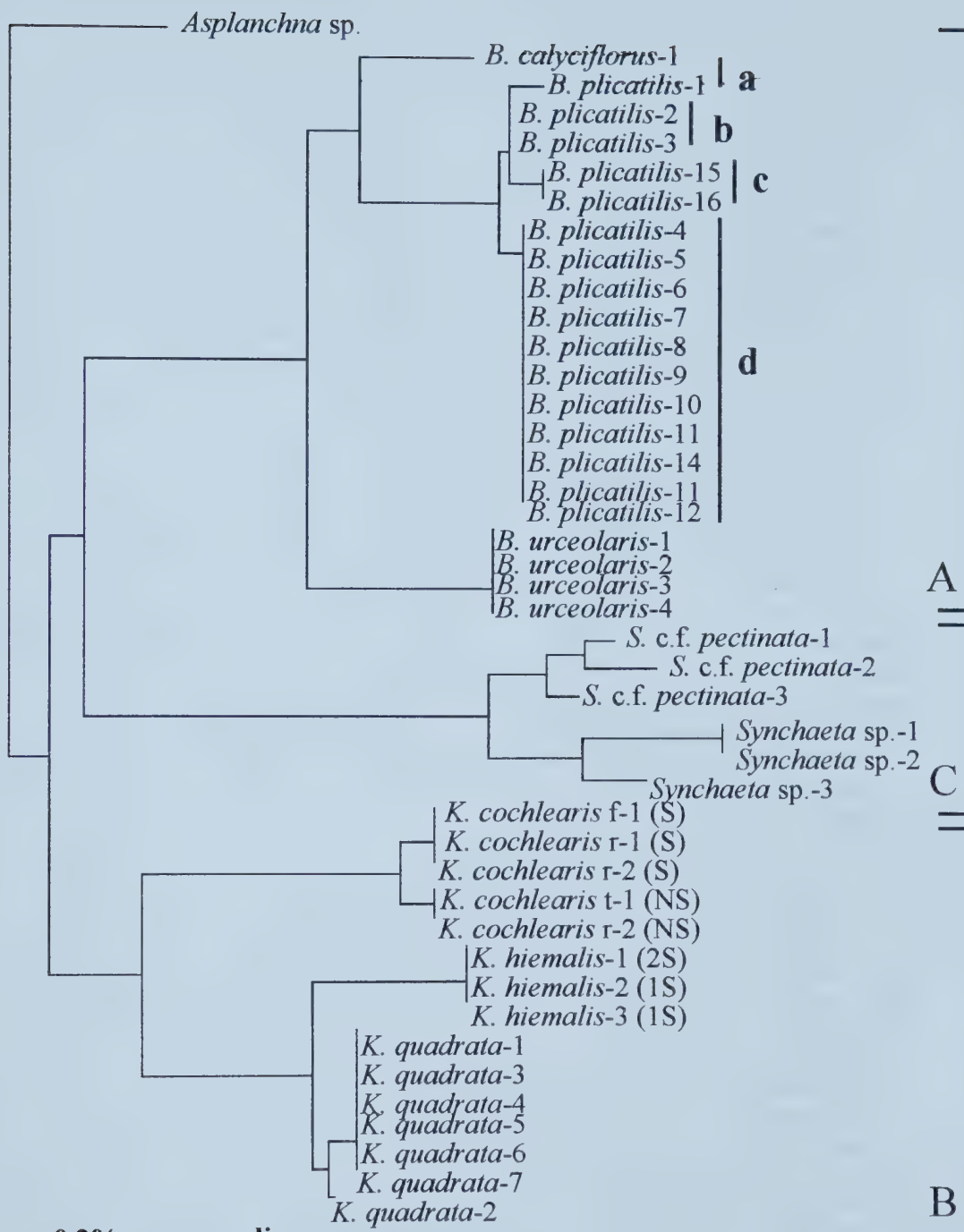
Appendix D. Scatter plot of transition/transversion (Ts/Tv) ratios versus percent sequence divergence for pairwise comparisons of COI nucleotide sequences isolated from rotifer species.



Appendix E. Neighbour-joining phenogram of 15 16S rotifer sequences based on the Kimura (1980) two-parameter model for distance correction and rooting with *Asplanchna* sp. Numbers at terminal branches give the haplotype and correspond with the numbering assigned to equivalent COI taxa. Capitalized letter clades indicate clusters representing different genera: A = *Brachionus* sp., B = *Keratella* sp., and C = *Synchaeta* sp. Pairwise divergences between taxa corrected by the Kimura (1980) two-parameter model are given in Table 3-2.

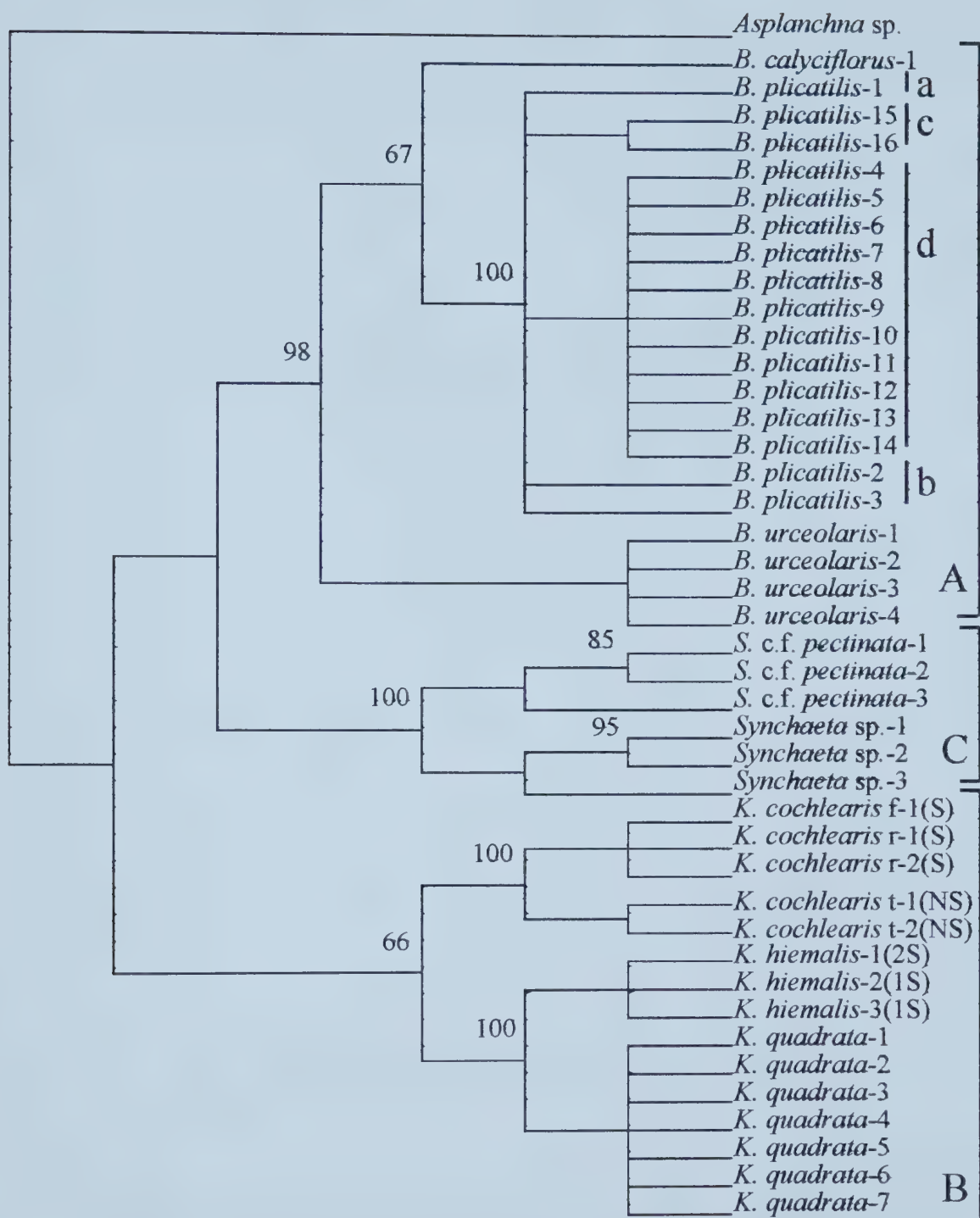


Appendix F. Neighbour-joining phenogram of 42 rotifer nucleotide sequences based on the Tamura-Nei (1993) model for distance correction and rooting with *Asplanchna* sp. Numbers at terminal branches give the haplotype and correspond with numbering assigned to equivalent 16S taxa. Capitalized letter clades indicate clusters representing different genera. Spined (S) and unspined (NS) subspecies of *Keratella cochlearis* as well as two-spined (2S) and one-spined morphs (1S) of *K. hiemalis* are indicated on the figure. Pairwise divergences between taxa corrected by the Tamura-Nei (1993) model are given in Table 3-4.

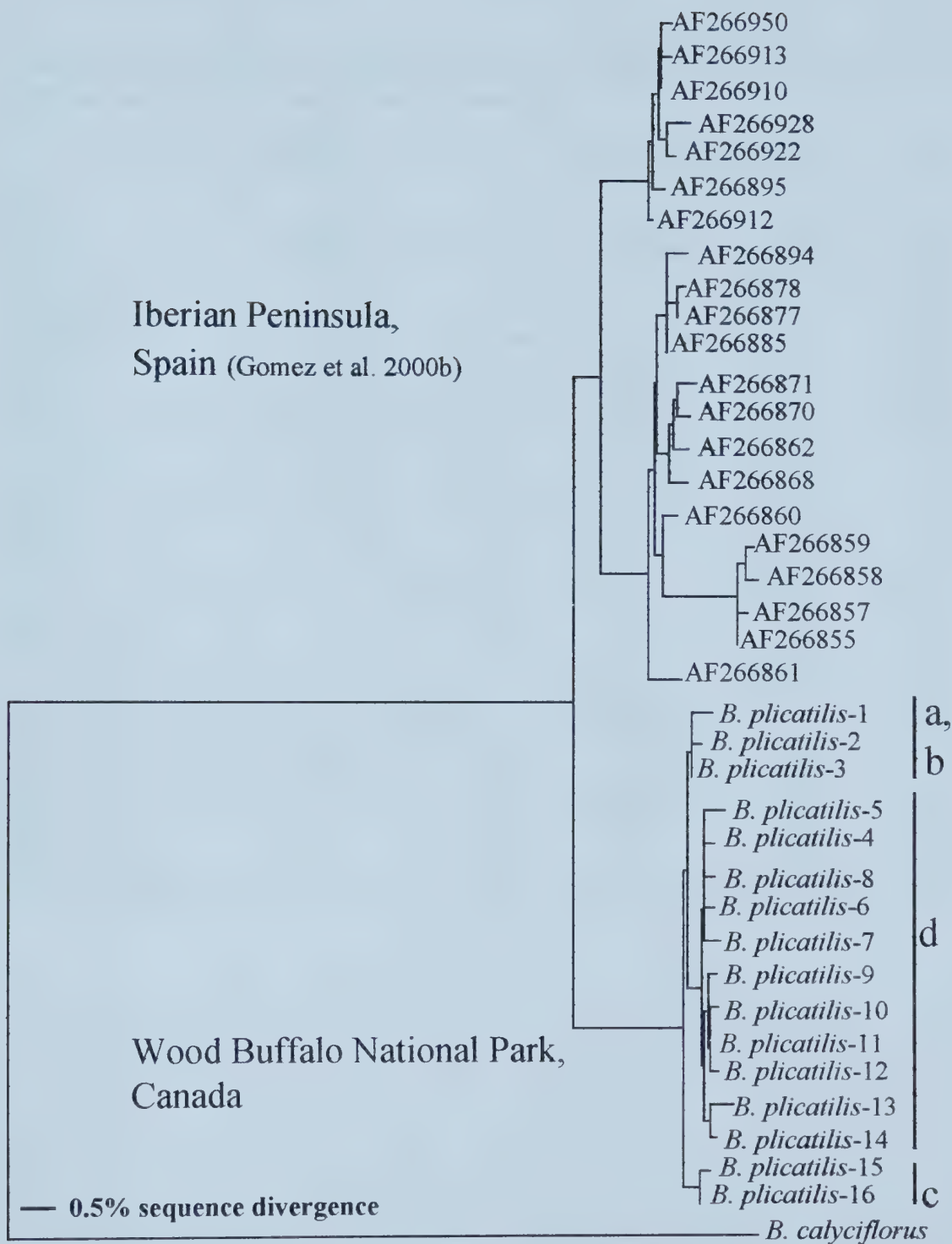


— 0.2% sequence divergence

Appendix G. Neighbour-joining phenogram of 42 COI rotifer amino acid sequences based on uncorrected p-distance and rooting with *Asplanchna* sp. Numbers at the terminal branches give the nucleotide haplotype and correspond with the numbering assigned to equivalent 16S taxa. Capitalized letter clades indicate clusters representing different genera: A = *Brachionus* sp., B = *Keratella* sp., and C = *Synchaeta* sp. Lowercase letter clades indicate clusters of *Brachionus plicatilis* amino acid haplotypes and correspond to the lakes from which the animals were collected: clade a and b = HC Lake, clade c = FP Lake, and clade d = GL/SPL Lake. Spined (S) and unspined (NS) subspecies of *Keratella cochlearis*, as well as two-spined (2S) and one-spined (1S) morphs of *K. hiemalis* are indicated on the figure. Pairwise divergences among taxa based on mean character differences are given in Table 3-4.



Appendix H. Most parsimonious tree of the 42 COI rotifer amino acid sequences rooted with *Asplanchna* sp. Bootstrap support (1000 pseudoreplicates) for major clades is indicated above the nodes. Numbers at the terminal branches give the nucleotide haplotypes and correspond with the numbering assigned to equivalent 16S taxa. Capitalized letter clades indicate clusters representing different genera: A = *Brachionus* sp., B = *Keratella* sp., and C = *Synchaeta* sp. Lowercase letter clades indicate clusters of *Brachionus plicatilis* amino acid haplotypes and correspond to the lakes from which the animals were collected: clade a and b: HC Lake, clade c = FP Lake, and clade d = GL/SPL Lake. Spined (S) and unspined (NS) subspecies of *Keratella cochlearis* as well as two-spined (2S) and one-spined (1S) morphs of *K. hiemalis* are indicated on the figure.



Appendix I. Neighbour-joining phenogram of 37 COI *Brachionus plicatilis* nucleotide sequences based on the Kimura (1980) two-parameter model and rooting with *B. calyciflorus*. Accession numbers AF266855 to AF266950 belong to the 21 haplotypes identified by Gomez et al. (2000b) on the Iberian Peninsula, Spain. *B. plicatilis* -1 to -16 are haplotypes found in Wood Buffalo National Park (WBNP), Canada. Numbers at the terminal branches give nucleotide haplotypes for WBNP populations and correspond with the numbering assigned on other 16S and COI trees. Lowercase letter clades indicate clusters of WBNP *B. plicatilis* that correspond to the lakes from which the animals were collected: clades a and b = HC Lake, clade c = FP Lake, and clade d = GL/SPL Lake. Pairwise divergences corrected by the Kimura (1980) two-parameter model between selected taxa are given in the text.

Appendix J. Chemical data for the study lakes, including chlorophyll *a* and secchi depth, on each sampling date given in Julian days.

	OL-SO4	PN-SO4	FL-SO4	SL-CI	SL-CI
Julian Day	157	156	157	160	232
TP (µg/l)	25,530.00	2,915.00	1,322.00	63.50	106.1
TN (µg/l)	862.22	47.15	61.63	1,238.77	1,707.07
NO ₂ +NO ₃ (µg/l)	-	-	-	-	9.98
DOC (mg/l)	290.13	71.80	75.20	19.41	21.41
Cl (mg/l)	856.04	279.99	170.73	3,190.00	4,474.47
SO ₄ (mg/l)	3,387.10	7,544.00	3,590.00	294.26	385.56
Na (mg/l)	37,048.00	4,898.00	2,857.00	2,122.00	2,861.00
K (mg/l)	606.80	127.80	79.30	6.08	8.26
Ca (mg/l)	0.63	4.02	2.84	136.70	155
Mg (mg/l)	45.60	94.70	58.30	56.20	71.9
Fe (mg/l)	2.04	0.19	1.23	0.22	0.08
Mn (mg/l)	0.13	0.02	0.03	0.10	0.1
Conductivity (µS/cm)	73,336.00	16,440.60	10,229.80	10,184.90	14,103.60
TDS (mg/l)	96,228.50	14,277.00	8,028.50	6,291.00	8,273.10
Colour (mg/l Pt)	67.30	35.00	96.70	31.70	35.4
Turbidity (NTU)	2.60	10.00	27.00	2.10	1.9
Alkalinity (mg/l CaCO ₃)	45,232.00	3,264.00	3,778.40	146.11	128.44
Alkalinity (mg/l HCO ₃)	9,377.00	2,666.00	3,024.00	178.15	156.6
Alkalinity (mg/l CO ₃)	22,508.00	646.00	778.00	-	-
Salinity (g/l)	62.8	7.6	4.6	4.4	2.9
Temperature (°C)	17.2	19.7	18.8	17	24.9
pH	10.2	9.55	9.31	8.13	8.38
Secchi depth (m)	0.65	0.55	0.3	0.41	0.32
Chlorophyll <i>a</i> (µg/l)	2.37	4.92	2.74	2.48	2.67

Appendix J. Chemical data for the study lakes, including chlorophyll *a* and secchi depth, on each sampling date given in Julian days (continued).

	GB-CI	GB-CI	GB-CI	GB-CI	SP-CI
Julian Day	184	204	220	261	191
TP (µg/l)	24.20	16.90	15.50	15.50	41.00
TN (µg/l)	859.91	804.18	691.4	609.51	1940.49
NO ₂ +NO ₃ (µg/l)	2.19	-	-	-	1.75
DOC (mg/l)	14.28	14.56	14.65	12.06	25.45
Cl (mg/l)	16,475.50	16,191.96	13,564.50	20,826.94	5,264.70
SO ₄ (mg/l)	1,070.42	1,321.17	1,282.70	1,162.51	2,213.20
Na (mg/l)	10,920.00	10,474.00	8,692.00	7,993.00	3,581.00
K (mg/l)	7.34	7.48	7.15	6.68	3.96
Ca (mg/l)	402.20	498.40	476.1	452.8	728.40
Mg (mg/l)	34.80	39.60	35.6	32.8	44.40
Fe (mg/l)	0.14	0.16	0.13	0.08	0.07
Mn (mg/l)	0.06	0.04	0.04	0.03	0.03
Conductivity (µS/cm)	44,514.60	43,587.00	37,919.20	34,848.00	17,574.00
TDS (mg/l)	29,410.90	29,104.10	24,506.50	22,252.00	11,764.10
Colour (mg/l Pt)	13.60	8.20	7.3	13.2	19.90
Turbidity (NTU)	1.60	0.85	1.1	1.9	6.60
Alkalinity (mg/l CaCO ₃)	69.12	55.18	53.49	57.58	50.81
Alkalinity (mg/l HCO ₃)	84.27	67.28	65.22	70.2	61.95
Alkalinity (mg/l CO ₃)	-	-	-	-	-
Salinity (g/l)	29.5	28.3	22.3	-	8.2
Temperature (°C)	18.5	17.6	21.2	11.4	28.9
pH	8.23	8.65	8.72	8	7.6
Secchi depth (m)	0.4	0.41	0.49	0.5	0.1
Chlorophyll <i>a</i> (µg/l)	5.43	1.61	2.78	2.96	9.01

Appendix J. Chemical data for the study lakes, including chlorophyll *a* and secchi depth, on each sampling date given in Julian days (continued).

	SP-CI	SP-CI	HC-CI	HC-CI	HC-CI
Julian Day	224	261	176	196	215
TP (µg/l)	28.6	18	72.90	81.00	52.20
TN (µg/l)	1,823.53	1,255.73	879.99	1,160.49	866.08
NO ₂ +NO ₃ (µg/l)	-	-	39.10	-	-
DOC (mg/l)	28.5	33.54	17.99	21.38	18.15
Cl (mg/l)	6,116.11	5,635.63	9,715.70	10,743.60	12,337.11
SO ₄ (mg/l)	2,321.55	2,122.56	1,053.00	1,120.95	1,257.83
Na (mg/l)	4,077.00	3,520.00	5,997.00	6,817	7,486.00
K (mg/l)	4.49	4.34	18.40	19.20	23
Ca (mg/l)	880.3	808.4	500.40	556.10	614.9
Mg (mg/l)	52.1	47.7	173.20	197.50	214.8
Fe (mg/l)	0.08	0.03	0.12	0.13	0.16
Mn (mg/l)	0.04	0.04	0.05	0.07	0.06
Conductivity (µS/cm)	20,370.20	18,833.20	27,398.00	30,800	33,667.20
TDS (mg/l)	13,981.50	12,282.00	19,230.40	21,221.00	23,206.60
Colour (mg/l Pt)	13.2	17.8	77.80	88.30	73.6
Turbidity (NTU)	1.5	1.9	6.60	2.00	2.7
Alkalinity (mg/l CaCO ₃)	48.54	62.83	91.00	92.08	93.05
Alkalinity (mg/l HCO ₃)	59.18	76.61	110.95	112.27	113.45
Alkalinity (mg/l CO ₃)	-	-	-	-	-
Salinity (g/l)	4.5	-	-	38.3	18.9
Temperature (°C)	19.1	9.5	-	32.2	24
pH	8.89	7.99	8.26	9.4	9.15
Secchi depth (m)	0.33	0.3	0.77	0.5	0.5
Chlorophyll <i>a</i> (µg/l)	5.21	3.77	10.6	11.12	8.02

Appendix J. Chemical data for the study lakes, including chlorophyll *a* and secchi depth, on each sampling date given in Julian days (continued).

	HC-Cl	GL-D	GL-D	GL-D	BP-D
Julian Day	246	190	223	257	188
TP (µg/l)	79.4	14.40	14.50	15.1	20.90
TN (µg/l)	778.73	654.69	602.43	603.21	982.41
NO ₂ +NO ₃ (µg/l)	-	47.30	-	-	26.10
DOC (mg/l)	15.44	10.89	10.43	10.31	24.49
Cl (mg/l)	19,551.50	27.61	28.6	31.9	148.20
SO ₄ (mg/l)	2,077.57	626.08	649.85	671.17	126.62
Na (mg/l)	11,852.00	27.90	31	33.1	65.40
K (mg/l)	34.6	2.72	2.88	2.93	3.71
Ca (mg/l)	985.7	176.80	193.4	209.2	55.90
Mg (mg/l)	340.4	43.20	46.8	51.5	40.60
Fe (mg/l)	0.17	0.01	-	-	0.03
Mn (mg/l)	0.06	-	0.01	0.01	0.01
Conductivity (µS/cm)	52,489.20	1,170.20	1,271.10	1,360.70	848.10
TDS (mg/l)	36,778.50	1,025.00	1,112.50	1,132.00	675.70
Colour (mg/l Pt)	45.5	8.60	6.5	13.6	78.70
Turbidity (NTU)	1.7	0.73	0.73	0.68	0.57
Alkalinity (mg/l CaCO ₃)	144.83	76.32	85.73	87.12	152.86
Alkalinity (mg/l HCO ₃)	176.58	93.07	104.53	106.22	183.17
Alkalinity (mg/l CO ₃)	-	-	-	-	1.58
Salinity (g/l)	-	0.7	0.7	-	0.6
Temperature (°C)	15.6	24	19.1	14.5	17.9
pH	8.39	8.07	8.25	7.91	8.7
Secchi depth (m)	0.47	3.33	3.5	3.87	1.72
Chlorophyll <i>a</i> (µg/l)	21.28	2.84	4.34	4.88	1.34

Appendix J. Chemical data for the study lakes, including chlorophyll *a* and secchi depth, on each sampling date given in Julian days (continued).

Julian Day	BP-D 217	BP-D 258	GW-D 176	GW-D 196	GW-D 214
TP (µg/l)	42.00	53	81.60	54.50	55.80
TN (µg/l)	1,071.23	1,086.07	1,048.95	1,246.24	1,095.15
NO2+NO3 (µg/l)	-	-	37.50	-	-
DOC (mg/l)	23.12	25.7	22.86	28.16	27.35
Cl (mg/l)	118.44	133.56	357.78	516.57	482.4
SO ₄ (mg/l)	77.4	66.48	14.45	23.79	15.47
Na (mg/l)	65.9	74.3	194.00	239.60	222.9
K (mg/l)	4.37	6.64	4.99	8.40	8.54
Ca (mg/l)	41.1	54.5	57.90	95.90	69.7
Mg (mg/l)	40	46.8	32.40	46.60	43.6
Fe (mg/l)	0.02	-	0.14	0.24	0.2
Mn (mg/l)	0.01	-	-	0.12	0.03
Conductivity (µS/cm)	783.7	955.3	1,477.40	1,930.00	1,793.60
TDS (mg/l)	556	593.5	19.10	1,356.00	1,290.00
Colour (mg/l Pt)	75.7	78.7	78.70	93.30	100.5
Turbidity (NTU)	0.86	1.3	1.80	1.80	1.3
Alkalinity (mg/l CaCO ₃)	148.27	208.79	106.26	139.28	110.14
Alkalinity (mg/l HCO ₃)	176.12	242.22	129.55	169.83	134.29
Alkalinity (mg/l CO ₃)	2.29	6.07	-	-	-
Salinity (g/l)	0.6	-	0.90	1	0.9
Temperature (°C)	18.9	13.5	-	16.3	17.6
pH	9.15	7.99	8.03	7.8	7.8
Secchi depth (m)	1.48	1.5	1.75	1.69	2
Chlorophyll <i>a</i> (µg/l)	4.91	14.56	6.32	1.42	1.42

Appendix J. Chemical data for the study lakes, including chlorophyll *a* and secchi depth, on each sampling date given in Julian days (continued).

	GW-D	FP-D	FP-D	FP-D	FP-D
Julian Day	252	181	203	216	256
TP (µg/l)	69.3	36.00	115.40	124.00	222.5
TN (µg/l)	1,210.75	1,173.30	1,823.19	1,324.94	1,758.70
NO ₂ +NO ₃ (µg/l)	-	2.53	-	-	-
DOC (mg/l)	29.02	32.73	41.88	39.22	40.88
Cl (mg/l)	503.99	146.50	241.20	227.13	372.28
SO ₄ (mg/l)	11.07	23.58	54.34	47.98	54.33
Na (mg/l)	245.2	69.50	123.30	116.1	200
K (mg/l)	10.1	3.25	3.62	3.35	3.99
Ca (mg/l)	84.3	48.20	74.40	72.6	88
Mg (mg/l)	49.6	29.80	41.80	40.2	45.2
Fe (mg/l)	0.49	0.12	0.77	0.34	0.07
Mn (mg/l)	0.45	-	0.12	0.02	0.02
Conductivity (µS/cm)	2,019.60	789.00	1,216.00	1,177.60	1,706.80
TDS (mg/l)	1,262.50	610.10	863.60	835	1,052.50
Colour (mg/l Pt)	103	189.40	243.50	203.7	186.9
Turbidity (NTU)	3.8	0.94	6.00	5.3	14
Alkalinity (mg/l CaCO ₃)	164.12	139.74	207.84	216.56	247.43
Alkalinity (mg/l HCO ₃)	194.38	170.39	243.40	255.17	288.12
Alkalinity (mg/l CO ₃)	2.82	-	4.92	4.36	6.67
Salinity (g/l)	-	0.60	0.7	0.6	-
Temperature (°C)	13.3	-	23.5	23	12.4
pH	7.56	7.7	7.78	8.15	8.15
Secchi depth (m)	1.47	1.39	0.64	0.51	0.28
Chlorophyll <i>a</i> (µg/l)	7.37	20.3	2.04	1.61	77.22

Appendix J. Chemical data for the study lakes, including chlorophyll *a* and secchi depth, on each sampling date given in Julian days (continued).

Julian Day	WR-D 179	WR-D 199	WR-D 217	WR-D 255
TP (µg/l)	27.50	29.10	32.50	36.9
TN (µg/l)	750.81	920.98	1,012.57	948.55
NO2+NO3 (µg/l)	4.26	-	-	-
DOC (mg/l)	18.72	22.55	22.87	24.15
Cl (mg/l)	163.72	163.59	182.91	201.96
SO ₄ (mg/l)	0.95	0.55	0.47	0.49
Na (mg/l)	66.30	69.90	72.3	85.4
K (mg/l)	5.90	5.96	5.83	6.44
Ca (mg/l)	33.00	36.40	39.9	47.3
Mg (mg/l)	27.40	29.60	33	39.8
Fe (mg/l)	0.32	0.15	0.05	0.07
Mn (mg/l)	0.01	-	-	0.01
Conductivity (µS/cm)	714.40	765.00	835.9	980.9
TDS (mg/l)	516.30	534.90	558.5	614.5
Colour (mg/l Pt)	71.90	108.00	96.7	93.3
Turbidity (NTU)	3.40	2.10	1.4	5.8
Alkalinity (mg/l CaCO ₃)	114.16	130.20	135.28	154.64
Alkalinity (mg/l HCO ₃)	139.19	158.75	164.94	182.25
Alkalinity (mg/l CO ₃)	-	-	-	3.09
Salinity (g/l)	0.40	0.6	0.3	
Temperature (°C)	-	18.9	16.5	12.7
PH	7.96	8.07	7.93	7.44
Secchi depth (m)	1.82	1.41	0.87	0.8
Chlorophyll <i>a</i> (µg/l)	4.16	2.3	2.24	5.69

Appendix K. Densities (# individuals/ l lake water) of rotifer species identified from the study lakes on each sampling date, given in Julian days.

	OL-S04	PN-S04	FL-S04	SL-CI
Julian Day	157	156	157	160
<i>Ascomorpha ecaudis</i>	0	0	0	0
<i>Ascomorpha ovalis</i>	0	0	0	0
<i>Anuraeopsis fissa</i>	0	0	0	0
<i>Asplanchna brightwelli</i>	0	0	0	0
<i>Asplanchna priodonta</i>	0	0	0	0
<i>Brachionus plicatilis</i>	0	0	0	4.4
<i>Brachionus quadridentatus</i>	0	0	0	0.6
<i>Brachionus rubens</i>	0	0	0	0
<i>Brachionus urceolaris</i>	0.02	26.3	6.6	0
<i>Collotheca mutabilis</i>	0	0	0	0
<i>Collotheca pelagica</i>	0	0	0	0
<i>Colurella obtusa</i>	0	0	0	0
<i>Colurella uncinata</i>	0	0	0	0
<i>Enicetruncus</i> sp.	0	0	0	0
<i>Filinia longiseta</i>	0	0	0	0
<i>Gastropus stylifer</i>	0	0	0	0
<i>Hexarthra</i> sp.	1.8	0	0	0.08
<i>Keratella cochlearis</i>	0	0.3	0.06	0
<i>Keratella hiemalis</i>	0	0	0	1.4
<i>Keratella quadrata</i>	0.02	0.1	0.03	19.0
<i>Keratella serrulata</i>	0	0	0	0
<i>Keratella testudo</i>	0	0.1	0.03	0
<i>Keratella ticinensis</i>	0	0	0	0
<i>Keratella valga</i>	0	0	0	0
<i>Lecane luna</i>	0	0	0	0
<i>Lecane ohioensis</i>	0	0	0	0
<i>Lepadella acuminata</i>	0	0	0	0
<i>Lepadella patella</i>	0	0	0	0
<i>Lophocharis salpina</i>	0	0	0	0
<i>Monostyla bulla</i>	0	0	0	0
<i>Monostyla closterocerca</i>	0	0	0	0
<i>Monostyla lunaris</i>	0	0	0	0
<i>Monostyla quadridentus</i>	0	0	0	0
<i>Mytilina ventralis</i> var. <i>brevispina</i>	0	0	0	0
<i>Notholca acuminata</i>	0	0	0	5.1
<i>Notommata</i> sp.	0	0	0	0
<i>Platyias patulus</i>	0	0	0	0
<i>Polyarthra dolichoptera</i>	0	0	0	0
<i>Polyarthra vulgaris</i>	0	0.4	0.10	0
<i>Pompholyx</i> sp.	0	0	0	0
<i>Synchaeta</i> sp.	0	0	0	0
<i>Testudinella patina</i>	0	0	0	0
<i>Trichocerca longiseta</i>	0	0	0	0
<i>Trichocerca lophoessa</i>	0	0	0	0
<i>Trichocerca multicrinis</i>	0	0	0	0
<i>Trichocerca rattus</i>	0	0	0	0
<i>Trichotria pocillum</i>	0	0	0	0
<i>Trichotria tetractis</i>	0	0	0	0
<i>Vanoyella globosa</i>	0	0	0	0

Appendix K. Densities (# individuals/l lake water) of rotifer species identified from the study lakes on each sampling date, given in Julian days (continued).

Julian Day	SL-CI 232	GB-CI 184	GB-CI 204	GB-CI 220
<i>Ascomorpha ecaudis</i>	0	0	0	0
<i>Ascomorpha ovalis</i>	0	0	0	0
<i>Anuraeopsis fissa</i>	0	0	0	0
<i>Asplanchna brightwelli</i>	0	0	0	0
<i>Asplanchna priodonta</i>	0	0	0	0
<i>Brachionus plicatilis</i>	0.4	869.4	575.2	106.2
<i>Brachionus quadridentatus</i>	1.1	0	0	0
<i>Brachionus rubens</i>	0.4	0	0	0
<i>Brachionus urceolaris</i>	0	0	0	0
<i>Collotheca mutabilis</i>	0	0	0	0
<i>Collotheca pelagica</i>	0	0	0	0
<i>Colurella obtusa</i>	0	0	0	0
<i>Colurella uncinata</i>	0	0	0	0
<i>Encentrum</i> sp.	0	0	0	0
<i>Filinia longiseta</i>	0	0	0	0
<i>Gastropus stylifer</i>	0	0	0	0
<i>Hexarthra</i> sp.	0	0	0	0.2
<i>Keratella cochlearis</i>	0	0	0	0
<i>Keratella hiemalis</i>	0	0	0	0
<i>Keratella quadrata</i>	0	0	0	0
<i>Keratella serrulata</i>	0	0	0	0
<i>Keratella testudo</i>	0	0	0	0
<i>Keratella ticinensis</i>	0	0	0	0
<i>Keratella valga</i>	0	0	0	0
<i>Lecane luna</i>	0	0	0	0
<i>Lecane ohioensis</i>	0	0	0	0
<i>Lepadella acuminata</i>	0	0	0	0
<i>Lepadella patella</i>	0	0	0	0
<i>Lophocharis salpina</i>	0	0	0	0
<i>Monostyla bulla</i>	0	0	0	0
<i>Monostyla closterocerca</i>	0	0	0	0
<i>Monostyla lunaris</i>	0	0	0	0
<i>Monostyla quadridentus</i>	0	0	0	0
<i>Mytilina ventralis</i> var. <i>brevispina</i>	0	0	0	0
<i>Notholca acuminata</i>	0.4	0	0	0
<i>Notommata</i> sp.	0	0	0	0
<i>Platylabus patulus</i>	0	0	0	0
<i>Polyarthra dolichoptera</i>	0	0	0	0
<i>Polyarthra vulgaris</i>	0	0	0	0
<i>Pompholyx</i> sp.	0	0	0	0
<i>Synchaeta</i> sp.	0	0	0	0
<i>Testudinella patina</i>	0	0	0	0
<i>Trichocerca longiseta</i>	0	0	0	0
<i>Trichocerca lophoessa</i>	0	0	0	0
<i>Trichocerca multicrinis</i>	0	0	0	0
<i>Trichocerca rattus</i>	0	0	0	0
<i>Trichotria pocillum</i>	0	0	0	0
<i>Trichotria tetractis</i>	0	0	0	0
<i>Vanoyella globosa</i>	0	0	0	0

Appendix K. Densities (# individuals/l lake water) of rotifer species identified from the study lakes on each sampling date, given in Julian days (continued).

	GB-CI	SP-CI	SP-CI	SP-CI
Julian Day	261	191	224	261
<i>Ascomorpha ecaudis</i>	0	0	0	0
<i>Ascomorpha ovalis</i>	0	0	0	0
<i>Anuraeopsis fissa</i>	0	0	0	0
<i>Asplanchna brightwelli</i>	0	0	0	0
<i>Asplanchna priodonta</i>	0	0	0	0
<i>Brachionus plicatilis</i>	106.2	65.3	202.2	82.8
<i>Brachionus quadridentatus</i>	0	0.8	0	0
<i>Brachionus rubens</i>	0	0	0	0
<i>Brachionus urceolaris</i>	0	0	0	0
<i>Collotheca mutabilis</i>	0	0	0	0
<i>Collotheca pelagica</i>	0	0	0	0
<i>Colurella obtusa</i>	0	0	0	0
<i>Colurella uncinata</i>	0	0	0	0
<i>Encentrum</i> sp.	0	0	0	0
<i>Filinia longiseta</i>	0	0	0	0
<i>Gastropus stylifer</i>	0	0	0	0
<i>Hexarthra</i> sp.	0.2	230.9	752.4	39.3
<i>Keratella cochlearis</i>	0	0	0	0
<i>Keratella hiemalis</i>	0	0	0	0
<i>Keratella quadrata</i>	0	0	0	0
<i>Keratella serrulata</i>	0	0	0	0
<i>Keratella testudo</i>	0	0	0	0
<i>Keratella ticinensis</i>	0	0	0	0
<i>Keratella valga</i>	0	0	0	0
<i>Lecane luna</i>	0	0	0	0
<i>Lecane ohioensis</i>	0	0	0	0
<i>Lepadella acuminata</i>	0	0	0	0
<i>Lepadella patella</i>	0	0	0	0
<i>Lophocharis salpina</i>	0	1.6	0	0
<i>Monostyla bulla</i>	0	0	0	0
<i>Monostyla closterocerca</i>	0	0	0	0
<i>Monostyla lunaris</i>	0	0	0	0
<i>Monostyla quadridentus</i>	0	0	0	0
<i>Mytilina ventralis</i> var. <i>brevispina</i>	0	0	0	0
<i>Notholca acuminata</i>	0	0	0	0
<i>Notommata</i> sp.	0	0	0	0
<i>Platylabus patulus</i>	0	0	0	0
<i>Polyarthra dolichoptera</i>	0	0	0	0
<i>Polyarthra vulgaris</i>	0	0	0	0
<i>Pompholyx</i> sp.	0	0	0	0
<i>Synchaeta</i> sp.	0	0	0	0
<i>Testudinella patina</i>	0	0	0	0
<i>Trichocerca longiseta</i>	0	0	0	0
<i>Trichocerca lophoessa</i>	0	0	0	0
<i>Trichocerca multirinis</i>	0	0	0	0
<i>Trichocerca ruttus</i>	0	0	0	0
<i>Trichotria pocillum</i>	0	0	0	0
<i>Trichotria tetractis</i>	0	0	0	0
<i>Vanoyella globosa</i>	0	0	0	0

Appendix K. Densities (# individuals/ l lake water) of rotifer species identified from the study lakes on each sampling date, given in Julian days (continued).

Julian Day	HC-CI 176	HC-CI 196	HC-CI 215	HC-CI 246
<i>Ascomorpha ecaudis</i>	0	0	0	0
<i>Ascomorpha ovalis</i>	0	0	0	0
<i>Anuraeopsis fissa</i>	0	0	0	0
<i>Asplanchna brightwelli</i>	0	0	0	0
<i>Asplanchna priodonta</i>	0	0	0	0
<i>Brachionus plicatilis</i>	32.6	11.5	34.3	486.9
<i>Brachionus quadridentatus</i>	0	0	0	0
<i>Brachionus rubens</i>	0	0	0	0
<i>Brachionus urceolaris</i>	0	0	0	0
<i>Collotheca mutabilis</i>	0	0	0	0
<i>Collotheca pelagica</i>	0	0	0	0
<i>Colurella obtusa</i>	0	0	0	0
<i>Colurella uncinata</i>	0	0	0	0
<i>Encentrum</i> sp.	0	0	0	0
<i>Filinia longiseta</i>	0	0	0	0
<i>Gastropus stylifer</i>	0	0	0	0
<i>Hexarthra</i> sp.	0	0.03	0	0
<i>Keratella cochlearis</i>	0	0.03	0	0
<i>Keratella hiemalis</i>	0	0	0	0
<i>Keratella quadrata</i>	0.7	2.7	0.03	0
<i>Keratella serrulata</i>	0	0	0	0
<i>Keratella testudo</i>	0	0	0	0
<i>Keratella ticinensis</i>	0	0	0	0
<i>Keratella valga</i>	0	0	0	0
<i>Lecane luna</i>	0	0	0	0
<i>Lecane ohioensis</i>	0	0	0	0
<i>Lepadella acuminata</i>	0	0	0	0
<i>Lepadella patella</i>	0	0	0	0
<i>Lophocharis salpina</i>	0	0	0	0
<i>Monostyla bulla</i>	0	0	0	0
<i>Monostyla closterocerca</i>	0	0	0	0
<i>Monostyla lunaris</i>	0	0	0	0
<i>Monostyla quadridentus</i>	0	0	0	0
<i>Mytilina ventralis</i> var. <i>brevispina</i>	0	0	0	0
<i>Notholca acuminata</i>	0	0.03	0	0
<i>Notommata</i> sp.	0	0	0	0
<i>Platytias patulus</i>	0	0	0	0
<i>Polyarthra dolichoptera</i>	0	0	0	0
<i>Polyarthra vulgaris</i>	0	0	0	0
<i>Pompholyx</i> sp.	0	0	0	0
<i>Synchaeta</i> sp.	0	0	0	0
<i>Testudinella patina</i>	0	0	0	0
<i>Trichocerca longiseta</i>	0	0	0	0
<i>Trichocerca lophoessa</i>	0	0	0	0
<i>Trichocerca multicrinis</i>	0	0	0	0
<i>Trichocerca rattus</i>	0	0	0	0
<i>Trichotria pocillum</i>	0	0	0	0
<i>Trichotria tetractis</i>	0	0	0	0
<i>Vanoyella globosa</i>	0	0	0	0

Appendix K. Densities (# individuals/l lake water) of rotifer species identified from the study lakes on each sampling date, given in Julian days (continued).

Julian Day	GL-D	GL-D	GL-D	BP-D
	190	223	257	188
<i>Ascomorpha ecaudis</i>	0	0.2	0.2	0
<i>Ascomorpha ovalis</i>	0	0.2	0	0
<i>Anuraeopsis fissa</i>	0	0	0	0
<i>Asplanchna brightwelli</i>	0	0	0	0
<i>Asplanchna priodonta</i>	427.8	2.7	3.3	0
<i>Brachionus plicatilis</i>	0	0	0	0
<i>Brachionus quadridentatus</i>	0	0	0	0
<i>Brachionus rubens</i>	0	0	0	0
<i>Brachionus urceolaris</i>	0	0	0	0
<i>Collotheca mutabilis</i>	19.1	0	3.6	0
<i>Collotheca pelagica</i>	2.1	0	0	0
<i>Colurella obtusa</i>	0	0	0	0
<i>Colurella uncinata</i>	0	0	0	7.5
<i>Encentrum</i> sp.	1.1	0	0	0
<i>Filinia longiseta</i>	204.9	21.8	117.6	0
<i>Gastropus stylifer</i>	38.2	1.8	3.8	0
<i>Hexarthra</i> sp.	1.1	0	0	0
<i>Keratella cochlearis</i>	348.2	21.6	37.8	0
<i>Keratella hiemalis</i>	108.3	0.2	0.9	0
<i>Keratella quadrata</i>	515.9	0.5	0	7.5
<i>Keratella serrulata</i>	0	0	0	0
<i>Keratella testudo</i>	0	0	0	7.5
<i>Keratella ticinensis</i>	0	0	0	0
<i>Keratella valga</i>	1.1	0	0	0
<i>Lecane luna</i>	1.1	0	0	7.5
<i>Lecane ohioensis</i>	0	0	0	0
<i>Lepadella acuminata</i>	0	0	0	0
<i>Lepadella patella</i>	0	0	0	0
<i>Lophocharis salpina</i>	2.1	0.2	0	0
<i>Monostyla bulla</i>	2.1	0	0	239.1
<i>Monostyla closteroerca</i>	0	0	0	0
<i>Monostyla lunaris</i>	0	0	0	0
<i>Monostyla quadridentus</i>	0	0	0	0
<i>Mytilina ventralis</i> var. <i>brevispina</i>	0	0	0	0
<i>Notholca acuminata</i>	2.1	0	0	0
<i>Notommata</i> sp.	0	0	0	0
<i>Platvias patulus</i>	0	0	0	0
<i>Polyarthra dolichoptera</i>	5.3	0	0.1	0
<i>Polyarthra vulgaris</i>	421.4	11.6	11.4	7.5
<i>Pompholyx</i> sp.	0	0	0	0
<i>Synchaeta</i> sp.	426.8	3.7	6.1	1740.9
<i>Testudinella patina</i>	0	0	0	7.5
<i>Trichocerca longiseta</i>	0	0	0	7.5
<i>Trichocerca lophoessa</i>	0	0	0	0
<i>Trichocerca multirinis</i>	60.5	0.5	2.6	0
<i>Trichocerca rattus</i>	0	0	0	0
<i>Trichotria pocillum</i>	0	0	0	7.5
<i>Trichotria tetractis</i>	0	0	0	0
<i>Vanoyella globosa</i>	0	0	0	0

Appendix K. Densities (# individuals/ l lake water) of rotifer species identified from the study lakes on each sampling date, given in Julian days (continued).

Julian Day	BP-D	BP-D	GW-D	GW-D
	217	258	176	196
<i>Ascomorpha ecaudis</i>	0	0	0	31.8
<i>Ascomorpha ovalis</i>	0	0	0	0
<i>Anuraeopsis fissa</i>	0	5.1	0	0
<i>Asplanchna brightwelli</i>	0	0.6	0	0
<i>Asplanchna priodonta</i>	0	0	0	0
<i>Brachionus plicatilis</i>	0	0	0	0
<i>Brachionus quadridentatus</i>	18.1	0	5.8	0
<i>Brachionus rubens</i>	0	0	0	0
<i>Brachionus urceolaris</i>	0	0	0	0
<i>Collotheca mutabilis</i>	0	0	0	0
<i>Collotheca pelagica</i>	0	0	0	0
<i>Colurella obtusa</i>	0	0	0	0
<i>Colurella uncinata</i>	0	0	0	0
<i>Encentrum</i> sp.	0	0	0	0
<i>Filinia longiseta</i>	0	0	0	0
<i>Gastropus stylifer</i>	0	0	0	0
<i>Hexarthra</i> sp.	0	1.3	0	0
<i>Keratella cochlearis</i>	72.4	776.6	11.7	6.4
<i>Keratella hiemalis</i>	0	0	5.8	6.4
<i>Keratella quadrata</i>	0	0	608.8	6732.5
<i>Keratella serrulata</i>	24.1	0	0	0
<i>Keratella testudo</i>	126.7	68.9	0	6.4
<i>Keratella ticinensis</i>	0	0.6	0	0
<i>Keratella valga</i>	0	0	0	0
<i>Lecane luna</i>	0	0	0	0
<i>Lecane ohioensis</i>	6.0	0	0	0
<i>Lepadella acuminata</i>	0	0	0	0
<i>Lepadella patella</i>	0	0	0	12.7
<i>Lophocharis salpina</i>	0	0	0	6.4
<i>Monostyla bulla</i>	12.1	0	41.0	12.7
<i>Monostyla closterocerca</i>	0	0	0	0
<i>Monostyla lunaris</i>	0	0.6	5.8	0
<i>Monostyla quadridentus</i>	0	0	0	0
<i>Mytilina ventralis</i> var. <i>brevispina</i>	48.2	0.6	52.7	0
<i>Notholca acuminata</i>	0	0	11.7	25.5
<i>Notommata</i> sp.	0	0	0	51.0
<i>Platylabus patulus</i>	12.1	0	0	0
<i>Polyarthra dolichoptera</i>	12.1	0.6	5.8	0
<i>Polyarthra vulgaris</i>	3130.4	85.3	58.5	19.1
<i>Pompholyx</i> sp.	0	33.5	0	19.1
<i>Synchaeta</i> sp.	2304.1	25.9	76.1	280.2
<i>Testudinella patina</i>	0	0	11.7	0
<i>Trichocerca longiseta</i>	0	0	0	0
<i>Trichocerca lophoessa</i>	0	0	0	0
<i>Trichocerca multicrinis</i>	0	0	0	0
<i>Trichocerca rattus</i>	0	0	0	0
<i>Trichotria pocillum</i>	0	0	0	0
<i>Trichotria tetractis</i>	0	0	0	6.4
<i>Vanoyella globosa</i>	0	0	0	0

Appendix K. Densities (# individuals/ l lake water) of rotifer species identified from the study lakes on each sampling date, given in Julian days (continued).

Julian Day	GW-D 214	GW-D 252	FP-D 181	FP-D 203
<i>Ascomorpha ecaudis</i>	89.2	6.4	0	0
<i>Ascomorpha ovalis</i>	0	0	0	0
<i>Anuraeopsis fissa</i>	0	25.5	0	0
<i>Asplanchna brightwelli</i>	6.4	0	0	0
<i>Asplanchna priodonta</i>	0	0	0	0
<i>Brachionus plicatilis</i>	0	0	2712.0	3.2
<i>Brachionus quadridentatus</i>	0	0	0	0
<i>Brachionus rubens</i>	0	0	0	0
<i>Brachionus urceolaris</i>	0	0	0	0
<i>Collotheca mutabilis</i>	0	0	0	0
<i>Collotheca pelagica</i>	0	0	0	0
<i>Colurella obtusa</i>	6.4	0	0	0
<i>Colurella uncinata</i>	0	0	0	0
<i>Encentrum</i> sp.	0	0	0	0
<i>Filinia longiseta</i>	0	0	0	0
<i>Gastropus styliifer</i>	0	0	0	0
<i>Hexarthra</i> sp.	0	0	0	0
<i>Keratella cochlearis</i>	0	248.4	31.3	0
<i>Keratella hiemalis</i>	0	0	0	0
<i>Keratella quadrata</i>	51.0	51.0	0	0
<i>Keratella serrulata</i>	0	19.1	0	0
<i>Keratella testudo</i>	12.7	0	0	0
<i>Keratella ticinensis</i>	0	0	0	0
<i>Keratella valga</i>	0	0	0	0
<i>Lecane luna</i>	12.7	6.4	0	0
<i>Lecane ohioensis</i>	0	0	0	0
<i>Lepadella acuminata</i>	0	0	0	0
<i>Lepadella patella</i>	6.4	0	0	0
<i>Lophocharis salpina</i>	0	0	0	0
<i>Monostyla bulla</i>	0	0	0	0
<i>Monostyla closteroerca</i>	6.4	0	0	0
<i>Monostyla lunaris</i>	12.7	0	0	0
<i>Monostyla quadridentus</i>	6.4	0	0	0
<i>Mytilina ventralis</i> var. <i>brevispina</i>	0	0	0	0
<i>Notholca acuminata</i>	6.4	0	0	0
<i>Notommata</i> sp.	0	0	0	0
<i>Platyias patulus</i>	0	0	6.3	0
<i>Polyarthra dolichoptera</i>	0	12.7	0	0
<i>Polyarthra vulgaris</i>	70.1	44.6	0	0
<i>Pompholyx</i> sp.	0	51.0	0	0
<i>Synchaeta</i> sp.	108.3	6.4	0	0
<i>Testudinella patina</i>	0	0	0	0
<i>Trichocerca longiseta</i>	0	0	0	0
<i>Trichocerca lophoessa</i>	0	0	0	0
<i>Trichocerca multirinis</i>	0	0	0	0
<i>Trichocerca rattus</i>	0	0	0	0
<i>Trichotria pocillum</i>	0	0	0	0
<i>Trichotria tetractis</i>	6.4	0	0	0
<i>Vanovella globosa</i>	0	0	0	0

Appendix K. Densities (# individuals/ l lake water) of rotifer species identified from the study lakes on each sampling date, given in Julian days (continued).

Julian Day	FP-D	FP-D	WR-D	WR-D
	216	256	179	199
<i>Ascomorpha ecaudis</i>	0	0	3.0	53.2
<i>Ascomorpha ovalis</i>	0	0	0	112.7
<i>Anuraeopsis fissa</i>	0	0	0	0
<i>Asplanchna brightwelli</i>	0	0	3.0	0
<i>Asplanchna priodonta</i>	0	0	0	0
<i>Brachionus plicatilis</i>	0	4.9	0	0
<i>Brachionus quadridentatus</i>	0	0	0	0
<i>Brachionus rubens</i>	0	0	0	0
<i>Brachionus urceolaris</i>	0	0	0	0
<i>Collotheca mutabilis</i>	0	0	0	0
<i>Collotheca pelagica</i>	0	0	0	0
<i>Colurella obtusa</i>	0	0	0	0
<i>Colurella uncinata</i>	0	0	0	0
<i>Encentrum</i> sp.	0	0	0	0
<i>Filinia longiseta</i>	0	0	0	0
<i>Gastropus styliifer</i>	0	0	3.0	25.0
<i>Hexarthra</i> sp.	0	0	0	0
<i>Keratella cochlearis</i>	0	0	1440.6	37.6
<i>Keratella hiemalis</i>	0	0	0	0
<i>Keratella quadrata</i>	12.7	0	18.3	9.4
<i>Keratella serrulata</i>	0	0	0	0
<i>Keratella testudo</i>	0	0	0	0
<i>Keratella ticinensis</i>	0	0	0	0
<i>Keratella valga</i>	0	0	0	0
<i>Lecane luna</i>	0	0	0	0
<i>Lecane ohioensis</i>	0	0	0	0
<i>Lepadella acuminata</i>	0	0	0	0
<i>Lepadella patella</i>	0	0	0	0
<i>Lophocharis salpina</i>	0	0	0	0
<i>Monostyla bulla</i>	0	0	0	0
<i>Monostyla closterocerca</i>	0	0	0	0
<i>Monostyla lunaris</i>	0	0	0	3.1
<i>Monostyla quadridentus</i>	0	0	0	0
<i>Mytilina ventralis</i> var. <i>brevispina</i>	0	0	0	6.3
<i>Notholca acuminata</i>	0	0	0	0
<i>Notommata</i> sp.	0	0	0	9.4
<i>Polyarthra dolichoptera</i>	0	0	3.0	0
<i>Polyarthra vulgaris</i>	0	4.9	137.3	328.8
<i>Platylas patulus</i>	0	0	0	0
<i>Pompholyx</i> sp.	0	0	0	0
<i>Synchaeta</i> sp.	0	0	33.6	0
<i>Testudinella patina</i>	0	0	0	0
<i>Trichocerca longiseta</i>	0	0	0	0
<i>Trichocerca lophoessa</i>	0	0	3.0	0
<i>Trichocerca multirinis</i>	0	0	0	0
<i>Trichocerca rattus</i>	0	0	0	0
<i>Trichotria pocillum</i>	0	0	0	0
<i>Trichotria tetractis</i>	0	0	0	0
<i>Vanoyella globosa</i>	0	0	3.0	0

Appendix K. Densities (# individuals/l lake water) of rotifer species identified from the study lakes on each sampling date, given in Julian days (continued).

	WR-D	WR-D
Julian Day	217	255
<i>Ascomorpha ecaudis</i>	43.9	6.0
<i>Ascomorpha ovalis</i>	0	6.0
<i>Anuraeopsis fissa</i>	0	11.9
<i>Asplanchna brightwelli</i>	0	0
<i>Asplanchna priodonta</i>	0	0
<i>Brachionus plicatilis</i>	0	0
<i>Brachionus quadridentatus</i>	0	0
<i>Brachionus rubens</i>	0	0
<i>Brachionus urceolaris</i>	0	0
<i>Collotheca mutabilis</i>	0	0
<i>Collotheca pelagica</i>	0	0
<i>Colurella obtusa</i>	0	0
<i>Colurella uncinata</i>	0	0
<i>Encentrum</i> sp.	0	0
<i>Filinia longiseta</i>	0	1020.0
<i>Gastropus stylifer</i>	102.4	18.0
<i>Hexarthra</i> sp.	0	3.0
<i>Keratella cochlearis</i>	1389.0	212.3
<i>Keratella hiemalis</i>	0	0
<i>Keratella quadrata</i>	29.2	245.2
<i>Keratella serrulata</i>	0	12.0
<i>Keratella testudo</i>	9.8	1866.0
<i>Keratella ticinensis</i>	0	0
<i>Keratella valga</i>	0	0
<i>Lecane luna</i>	0	0
<i>Lecane ohioensis</i>	0	0
<i>Lepadella acuminata</i>	5.0	9.0
<i>Lepadella patella</i>	0	0
<i>Lophocharis salpina</i>	0	3.0
<i>Monostyla bulla</i>	0	0
<i>Monostyla closterocerca</i>	0	6.0
<i>Monostyla lunaris</i>	0	12.0
<i>Monostyla quadridentus</i>	0	0
<i>Mytilina ventralis</i> var. <i>brevispina</i>	4.9	6.0
<i>Notholca acuminata</i>	0	3.0
<i>Notommata</i> sp.	0	0
<i>Platyias patulus</i>	0	0
<i>Polyarthra dolichoptera</i>	4.9	3.0
<i>Polyarthra vulgaris</i>	1062.4	765.4
<i>Pompholyx</i> sp.	0	732.6
<i>Synchaeta</i> sp.	160.8	230.2
<i>Testudinella patina</i>	0	0
<i>Trichocerca longiseta</i>	0	0
<i>Trichocerca lophoessa</i>	0	0
<i>Trichocerca multicrinis</i>	0	0
<i>Trichocerca rattus</i>	4.9	0
<i>Trichotria pocillum</i>	0	9.0
<i>Trichotria tetractis</i>	0	0
<i>Vanoyella globosa</i>	0	0

Appendix L. Densities (# individuals/l lake water) of crustacean zooplankton species identified from the study lakes on each sampling date, given in Julian days.

	OL-SO4	PN-SO4	FL-SO4	SL-CI
Julian Day	157	156	157	160
<i>Agalodiaptomus leptopus</i>	0	0	0	0
<i>Diaptomus arcticus</i>	0	0	0	0
<i>Diaptomus nevadensis</i>	0	0.4	0.10	0
<i>Leptodiaptomus nudus</i>	0	0	0	0.1
<i>Leptodiaptomus sicilis</i>	0	5.9	1.5	0
Calanoid juveniles	0	4.6	1.2	6.3
<i>Acanthocyclops carolinianus</i>	0	0	0	0
<i>Acanthocyclops robustus</i>	0	0	0	0
<i>Acanthocyclops venustoide</i>	0	0	0	0
<i>Acanthocyclops vernalis</i>	0	0	0	0
<i>Diaicyclops navus</i>	0	0	0	0.5
Cyclopoid juveniles	0	0	0	27.3
<i>Cletocamptus</i> sp.	0	0	0	0
<i>Artemia franciscana</i>	27.9	0	0	0
<i>Alona circumfimbriata</i>	0	0	0	0
<i>Alona costata</i>	0	0	0	0
<i>Alona guttata</i>	0	0	0	0
<i>Alona rectangula</i>	0	0	0	0.2
<i>Bosmina</i> sp.	0	0	0	0
<i>Ceriodaphnia laticaudata</i>	0	0	0	0
<i>Ceriodaphnia pulchella</i>	0	0	0	3.7
<i>Chydorus brevilabris</i>	0	0	0	0
<i>Chydorus piger</i>	0	0	0	0
<i>Chydorus sphaericus</i>	0	0	0	0
<i>Daphnia parvula</i>	0	0	0	0
<i>Daphnia pulicaria/pulex</i>	0	0	0	6.4
<i>Daphnia rosea</i>	0	0	0	0
<i>Daphnia schoedleri</i>	0	0	0	0
<i>Polyphemus pediculus</i>	0	0	0	0

Appendix L. Densities (# individuals/ l lake water) of crustacean zooplankton species identified from the study lakes on each sampling date, given in Julian days (continued).

	SL-CI	GB-CI	GB-CI	GB-CI
Julian Day	232	184	204	220
<i>Agalodiaptomus leptopus</i>	0	0	0	0
<i>Diaptomus arcticus</i>	0	0	0	0
<i>Diaptomus nevadensis</i>	0	0	0	0
<i>Leptodiaptomus nudus</i>	17.6	0	0	0
<i>Leptodiaptomus sicilis</i>	0	0	0	0
Calanoid juveniles	73.6	0	0	0
<i>Acanthocyclops carolinianus</i>	0	0	0	0
<i>Acanthocyclops robustus</i>	0	0	0	0
<i>Acanthocyclops venustoide</i>	0	0	0	0
<i>Acanthocyclops vernalis</i>	0	0	0	0
<i>Diacyclops navus</i>	0.4	0	0	0
Cyclopoid juveniles	5.1	0	0	0
<i>Cletocamptus</i> sp.	0	61.1	3.8	0.5
<i>Artemia franciscana</i>	0	0	0	0
<i>Alona circumfimbriata</i>	0	0	0	0
<i>Alona costata</i>	0	0	0	0
<i>Alona guttata</i>	0	0	0	0
<i>Alona rectangula</i>	0	0	0	0
<i>Bosmina</i> sp.	0	0	0	0
<i>Ceriodaphnia laticaudata</i>	0	0	0	0
<i>Ceriodaphnia pulchella</i>	0	0	0	0
<i>Chydorus brevilabris</i>	0	0	0	0
<i>Chydorus piger</i>	0	0	0	0
<i>Chydorus sphaericus</i>	0	0	0	0
<i>Daphnia parvula</i>	0	0	0	0
<i>Daphnia pulicaria/pulex</i>	0	0	0	0
<i>Daphnia rosea</i>	0	0	0	0
<i>Daphnia schoedleri</i>	0	0	0	0
<i>Polyphemus pediculus</i>	0	0	0	0

Appendix L. Densities (# individuals/ l lake water) of crustacean zooplankton species identified from the study lakes on each sampling date, given in Julian days (continued).

	GB-CI	SP-CI	SP-CI	SP-CI
Julian Day	261	191	224	261
<i>Agalodiaptomus leptopus</i>	0	0	0	0
<i>Diaptomus arcticus</i>	0	0	0	0
<i>Diaptomus nevadensis</i>	0	0	0	0
<i>Leptodiaptomus nudus</i>	0	0	0	0
<i>Leptodiaptomus sicilis</i>	0	0	0	0
Calanoid juveniles	0	0	0	0
<i>Acanthocyclops carolinianus</i>	0	0.80	0	0
<i>Acanthocyclops robustus</i>	0	0	0	0
<i>Acanthocyclops venustoides</i>	0	0	0	0
<i>Acanthocyclops vernalis</i>	0	0	0	0
<i>Diacyclops navus</i>	0	0	0	0
Cyclopoid juveniles	0	19.9	178.1	35.3
<i>Cletocampus</i> sp.	0	0.80	0	0
<i>Artemia franciscana</i>	0	0	0	0
<i>Alona circumfimbriata</i>	0	0	0	0
<i>Alona costata</i>	0	0	0	0
<i>Alona guttata</i>	0	0	0	0
<i>Alona rectangula</i>	0	0	0	0
<i>Bosmina</i> sp.	0	0	0	0
<i>Ceriodaphnia laticaudata</i>	0	0	0	0
<i>Ceriodaphnia pulchella</i>	0	0	0	0
<i>Chydorus brevilabris</i>	0	0	0	0
<i>Chydorus piger</i>	0	0	0	0
<i>Chydorus sphaericus</i>	0	0	0	0
<i>Daphnia parvula</i>	0	0	0	0
<i>Daphnia pulicaria/pulex</i>	0	0	0	0
<i>Daphnia rosea</i>	0	0	0	0
<i>Daphnia schoedleri</i>	0	0	0	0
<i>Polyphemus pediculus</i>	0	0	0	0

Appendix L. Densities (# individuals/ l lake water) of crustacean zooplankton species identified from the study lakes on each sampling date, given in Julian days (continued).

Julian Day	HC-CI 176	HC-CI 196	HC-CI 215	HC-CI 246
<i>Agalodiaptomus leptopus</i>	0	0	0	0
<i>Diaptomus arcticus</i>	0	0	0	0
<i>Diaptomus nevadensis</i>	0	0	0	0
<i>Leptodiaptomus nudus</i>	0	0	0	0
<i>Leptodiaptomus sicilis</i>	0	0	0	0
Calanoid juveniles	0	0.05	0	0
<i>Acanthocyclops carolinianus</i>	0	0	0	0
<i>Acanthocyclops robustus</i>	0	0	0	0
<i>Acanthocyclops venustoide</i>	0	0	0	0
<i>Acanthocyclops vernalis</i>	0	0	0	0
<i>Diacyclops navus</i>	0	0	0	0
Cyclopoid juveniles	0	0.5	0.03	0
<i>Cletocamptus</i> sp.	0.4	0	0.2	0
<i>Artemia franciscana</i>	0	0	0	0
<i>Alona circumfimbriata</i>	0	0	0	0
<i>Alona costata</i>	0	0	0	0
<i>Alona guttata</i>	0	0	0	0
<i>Alona rectangula</i>	0	0	0	0
<i>Bosmina</i> sp.	0	0.03	0	0
<i>Ceriodaphnia laticaudata</i>	0	0	0	0
<i>Ceriodaphnia pulchella</i>	0	0	0	0
<i>Chydorus brevilabris</i>	0	0	0	0
<i>Chydorus piger</i>	0	0	0	0
<i>Chydorus sphaericus</i>	0	0	0	0
<i>Daphnia parvula</i>	0	0.03	0	0
<i>Daphnia pulicaria/pulex</i>	0	0	0	0
<i>Daphnia rosea</i>	0	0	0	0
<i>Daphnia schoedleri</i>	0	0	0	0
<i>Polyphemus pediculus</i>	0	0	0	0

Appendix L. Densities (# individuals/ l lake water) of crustacean zooplankton species identified from the study lakes on each sampling date, given in Julian days (continued).

Julian Day	GL-D 190	GL-D 209	GL-D 257	BP-D 188
<i>Agalodiaptomus leptopus</i>	0	0	0	0
<i>Diaptomus arcticus</i>	0	0	0	0
<i>Diaptomus nevadensis</i>	0	0	0	0
<i>Leptodiaptomus nudus</i>	0	0	0	0
<i>Leptodiaptomus sicilis</i>	0	0.2	0	0
Calanoid juveniles	7.4	0	0	0
<i>Acanthocyclops carolinianus</i>	0	0	0	0
<i>Acanthocyclops robustus</i>	0	0	0	0
<i>Acanthocyclops venustoide</i>	0	0	0	0
<i>Acanthocyclops vernalis</i>	0	0	0	0
<i>Diacyclops navus</i>	0	0	0	0
Cyclopoid juveniles	1145.4	45.5	12.0	209.2
<i>Cletocamptus</i> sp.	0	0	0	0
<i>Artemia franciscana</i>	0	0	0	0
<i>Alona circumfimbriata</i>	0	0	0	0
<i>Alona costata</i>	0	0	0	0
<i>Alona guttata</i>	0	0	0	0
<i>Alona rectangula</i>	0	0	0	0
<i>Bosmina</i> sp.	0	0	0	0
<i>Ceriodaphnia laticaudata</i>	0	0	0	0
<i>Ceriodaphnia pulchella</i>	0	0	0	0
<i>Chydorus brevilabris</i>	0	0	0	0
<i>Chydorus piger</i>	0	0	0	0
<i>Chydorus sphaericus</i>	0	0	0	0
<i>Daphnia parvula</i>	0	0	0	0
<i>Daphnia pulicaria/pulex</i>	0	0	0	0
<i>Daphnia rosea</i>	0	0	0	0
<i>Daphnia schoedleri</i>	0	0	0	0
<i>Polyphemus pediculus</i>	0	0	0	0

Appendix L. Densities (# individuals/l lake water) of crustacean zooplankton species identified from the study lakes on each sampling date, given in Julian days (continued).

	BP-D	BP-D	GW-D	GW-D
Julian Day	217	258	176	196
<i>Agalodiaptomus leptopus</i>	0	0	0	0
<i>Diaptomus arcticus</i>	0	0	0	0
<i>Diaptomus nevadensis</i>	0	0	0	0
<i>Leptodiaptomus nudus</i>	0	0	0	0
<i>Leptodiaptomus sicilis</i>	0	0	0	0
Calanoid juveniles	0	0	128.8	324.8
<i>Acanthocyclops carolinianus</i>	0	0	0	0
<i>Acanthocyclops robustus</i>	0	0	0	0
<i>Acanthocyclops venustoides</i>	0	0	0	0
<i>Acanthocyclops vernalis</i>	0	0	0	0
<i>Diaicyclops navus</i>	0	0	0	0
Cyclopoid juveniles	72.4	5.7	989.4	2815.3
<i>Cletocamptus</i> sp.	0	0	0	0
<i>Artemia franciscana</i>	0	0	0	0
<i>Alona circumfimbriata</i>	0	0	0	0
<i>Alona costata</i>	0	0	0	0
<i>Alona guttata</i>	0	0	0	0
<i>Alona rectangula</i>	0	0	0	0
<i>Bosmina</i> sp.	0	0	0	0
<i>Ceriodaphnia laticaudata</i>	0	0	0	0
<i>Ceriodaphnia pulchella</i>	0	0	0	0
<i>Chydorus brevilabris</i>	0	0	0	0
<i>Chydorus piger</i>	0	1.3	0	19.1
<i>Chydorus sphaericus</i>	0	0	11.7	0
<i>Daphnia parvula</i>	0	0	35.1	38.2
<i>Daphnia pulicaria/pulex</i>	0	0	29.3	12.7
<i>Daphnia rosea</i>	0	0	29.3	0
<i>Daphnia schoedleri</i>	0	0	0	0
<i>Polyphemus pediculus</i>	0	0	0	0

Appendix L. Densities (# individuals/ l lake water) of crustacean zooplankton species identified from the study lakes on each sampling date, given in Julian days (continued).

Julian Day	GW-D 214	GW-D 252	FP-D 181	FP-D 203
<i>Agalodiaptomus leptopus</i>	0	0	18.8	9.7
<i>Diaptomus arcticus</i>	6.4	12.7	0	0
<i>Diaptomus nevadensis</i>	0	0	0	0
<i>Leptodiaptomus nudus</i>	0	0	0	0
<i>Leptodiaptomus sicilis</i>	0	0	0	0
Calanoid juveniles	522.3	19.1	181.6	161.9
<i>Acanthocyclops carolinianus</i>	0	0	0	0
<i>Acanthocyclops robustus</i>	0	0	0	0
<i>Acanthocyclops venustoide</i>	0	0	0	3.2
<i>Acanthocyclops vernalis</i>	0	0	0	3.2
<i>Diacyclops navus</i>	0	0	0	3.2
Cyclopoid juveniles	458.6	1242.0	739.1	430.6
<i>Cletocamptus</i> sp.	0	0	0	0
<i>Artemia franciscana</i>	0	0	0	0
<i>Alona circumfimbriata</i>	6.4	0	0	0
<i>Alona costata</i>	0	0	0	0
<i>Alona guttata</i>	0	0	0	0
<i>Alona rectangula</i>	0	0	0	0
<i>Bosmina</i> sp.	0	0	0	0
<i>Ceriodaphnia laticaudata</i>	0	76.4	0	0
<i>Ceriodaphnia pulchella</i>	0	0	0	0
<i>Chydorus brevilabris</i>	95.5	0	0	0
<i>Chydorus piger</i>	0	0	0	0
<i>Chydorus sphaericus</i>	19.1	0	0	0
<i>Daphnia parvula</i>	0	0	0	3.2
<i>Daphnia pulicaria/pulex</i>	101.9	38.2	206.6	262.3
<i>Daphnia rosea</i>	0	6.4	12.5	0
<i>Daphnia schoedleri</i>	0	0	0	0
<i>Polyphemus pediculus</i>	6.4	0	0	0

Appendix L. Densities (# individuals/ l lakewater) of crustacean zooplankton species identified from the study lakes on each sampling date, given in Julian days (continued).

Julian Day	FP-D 216	FP-D 256	WR-D 179	WR-D 199
<i>Agalodiaptomus leptopus</i>	25.5	68.3	0	0
<i>Diaptomus arcticus</i>	0	0	0	0
<i>Diaptomus nevadensis</i>	0	0	0	0
<i>Leptodiaptomus nudus</i>	0	0	0	0
<i>Leptodiaptomus sicilis</i>	0	0	0	0
<i>Calanoid juveniles</i>	598.7	0	149.6	109.6
<i>Acanthocyclops carolinianus</i>	0	0	0	0
<i>Acanthocyclops robustus</i>	0	0	0	0
<i>Acanthocyclops venustoide</i>	0	0	0	97.1
<i>Acanthocyclops vernalis</i>	0	4.9	0	0
<i>Diacyclops navus</i>	0	0	0	0
<i>Cyclopoid juveniles</i>	178.4	0	524.9	0
<i>Cletocamptus</i> sp.	0	0	0	0
<i>Artemia franciscana</i>	0	0	0	0
<i>Alona circumfimbriata</i>	0	0	0	0
<i>Alona costata</i>	0	0	3.0	0
<i>Alona guttata</i>	0	0	0	0
<i>Alona rectangula</i>	0	0	0	0
<i>Bosmina</i> sp.	0	0	0	0
<i>Ceriodaphnia laticaudata</i>	0	0	0	0
<i>Ceriodaphnia pulchella</i>	0	0	0	0
<i>Chydorus brevilabris</i>	0	0	0	0
<i>Chydorus piger</i>	0	0	0	0
<i>Chydorus sphaericus</i>	0	0	3.0	6.3
<i>Daphnia parvula</i>	0	0	0	0
<i>Daphnia pulicaria/pulex</i>	51.0	0	106.8	0
<i>Daphnia rosea</i>	0	0	70.2	3.1
<i>Daphnia schoedleri</i>	31.8	19.5	0	0
<i>Polyphemus pediculus</i>	0	0	0	0

Appendix L. Densities (# individuals/l lakewater) of crustacean zooplankton species identified from the study lakes on each sampling date, given in Julian days (continued).

Julian Day	WR-D 217	WR-D 255
<i>Agalodiaptomus leptopus</i>	0	0
<i>Diaptomus nevadensis</i>	0	0
<i>Diaptomus arcticus</i>	0	0
<i>Leptodiaptomus nudus</i>	0	0
<i>Leptodiaptomus sicilis</i>	0	0
Calanoid juveniles	9.8	6.0
<i>Acanthocyclops carolinianus</i>	0	0
<i>Acanthocyclops robustus</i>	14.6	0
<i>Acanthocyclops venustoide</i>	0	0
<i>Acanthocyclops vernalis</i>	0	0
<i>Diacyclops navus</i>	0	0
Cyclopoid juveniles	282.7	290.0
<i>Cletocamptus</i> sp.	0	0
<i>Artemia franciscana</i>	0	0
<i>Alona circumfimbriata</i>	0	0
<i>Alona costata</i>	0	0
<i>Alona guttata</i>	4.9	0
<i>Alona rectangula</i>	0	0
<i>Bosmina</i> sp.	0	0
<i>Ceriodaphnia laticaudata</i>	0	0
<i>Ceriodaphnia pulchella</i>	0	0
<i>Chydorus brevilabris</i>	0	0
<i>Chydorus piger</i>	0	0
<i>Chydorus sphaericus</i>	0	9.0
<i>Daphnia parvula</i>	0	0
<i>Daphnia pulicaria pulex</i>	0	0
<i>Daphnia rosea</i>	0	0
<i>Daphnia schoedleri</i>	0	0
<i>Polyphemus pediculus</i>	0	0

University of Alberta Library



0 1620 1492 0456

B45432